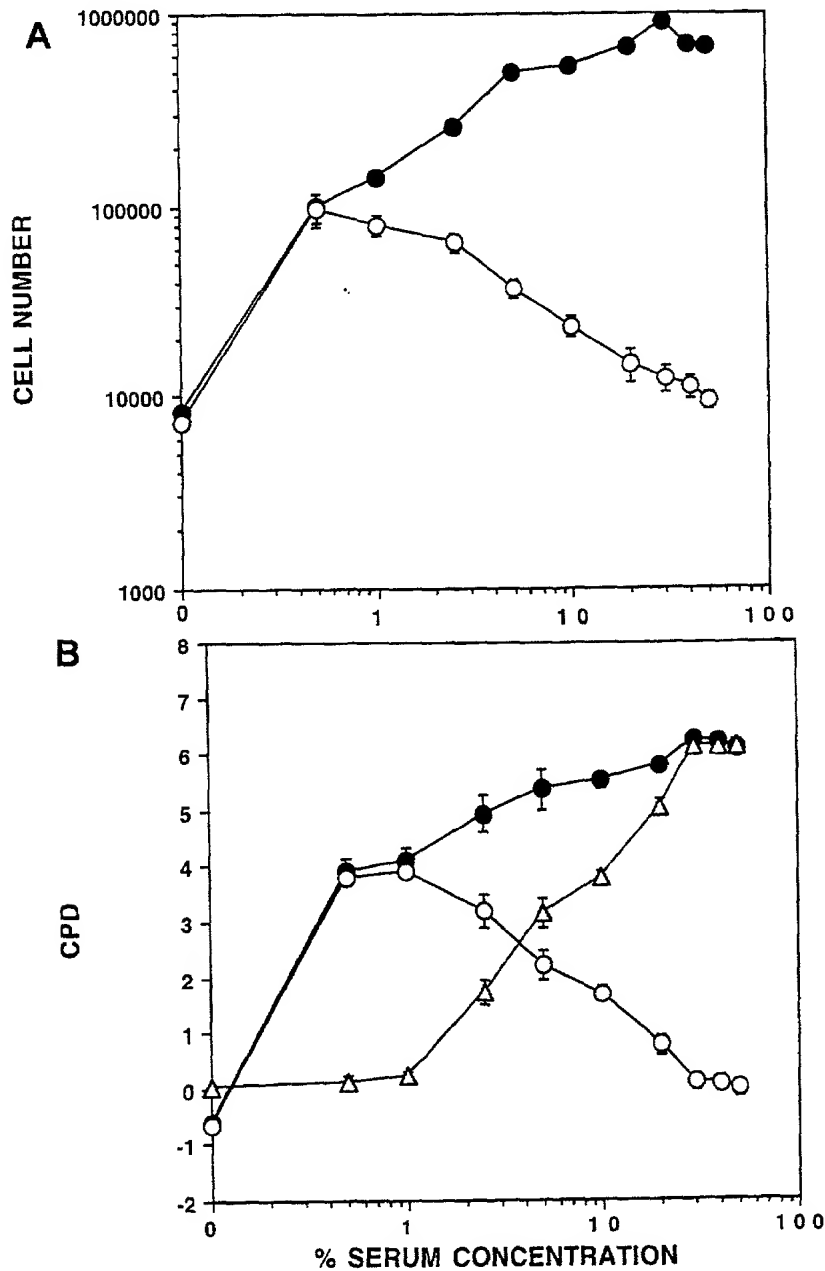


# FIGURE 1

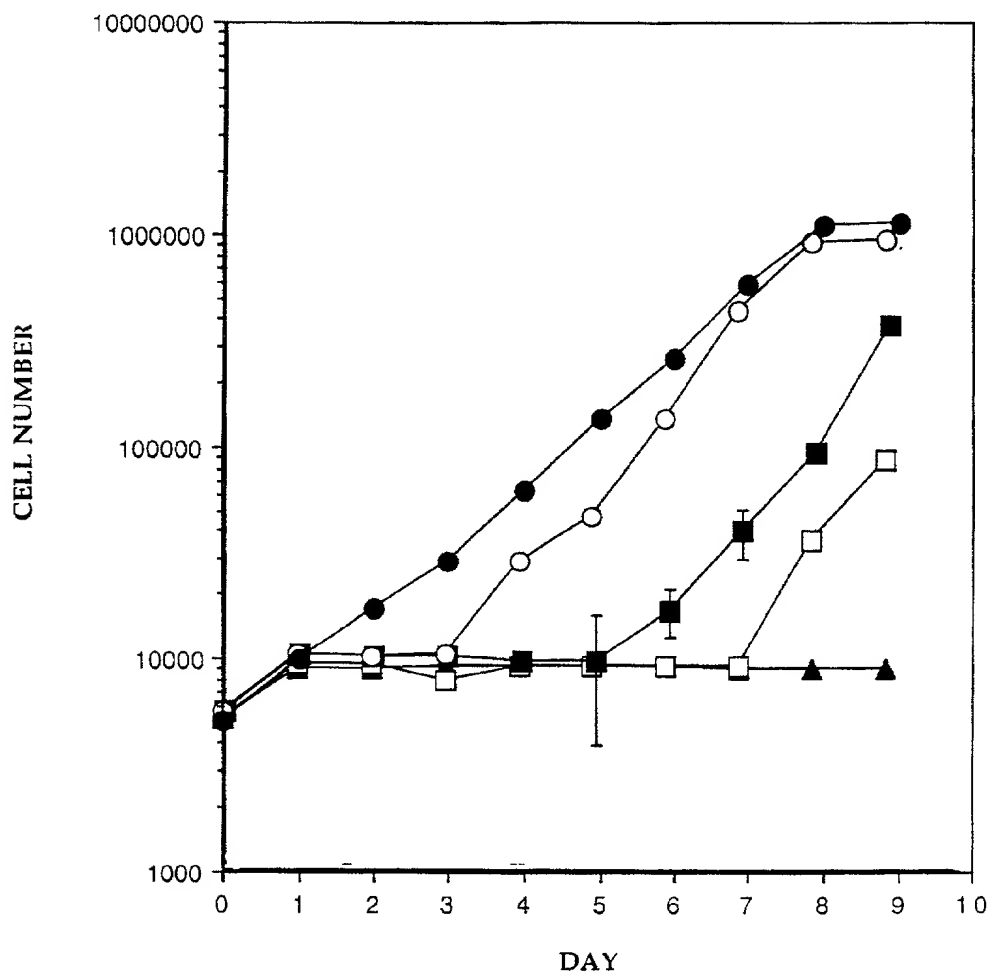
## MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM



- A: DATA EXPRESSED AS CELL NUMBER AFTER 7 DAYS**  
 Growth with  $1.0 \times 10^{-8} M E_2$  (closed circles) and without hormone (open circles) in medium containing the designated concentrations of serum.
- B. DATA IN (A) EXPRESSED AS CPD**  
 The symbols indicate the same conditions as (A) except the open triangles show CPD differences between growth in dishes with and without the hormone (Difference = estrogenic effect on growth).

**FIGURE 2**

**MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM WITH  
ESTROGENS ADDED AT VARIOUS TIMES AFTER SEEDING**

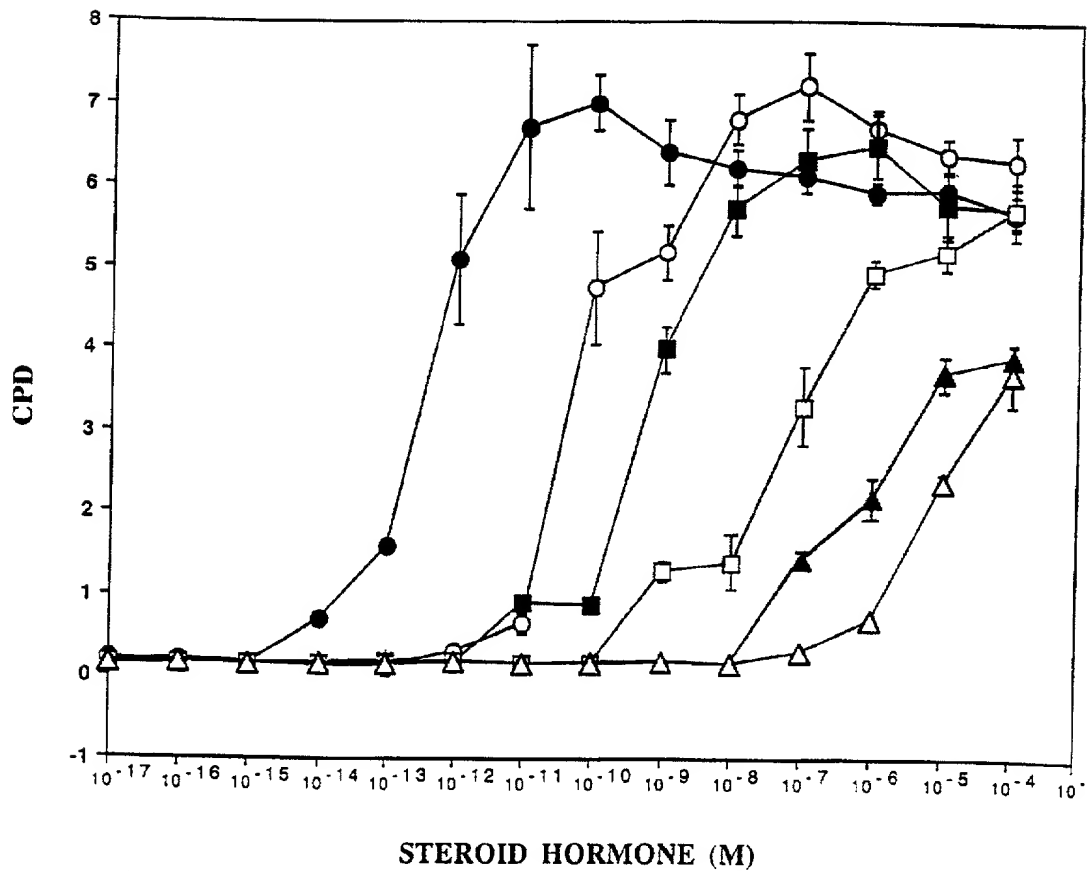


**LEGEND:**

Control growth in the absence of exogenous estrogen is shown by (triangles). In other dishes,  $1.0 \times 10^{-8}$  M  $E_2$  was added at the beginning of the experiment (closed circles), after 48 h (open circles), after 96 h (closed squares), or after 144 h (open squares).

**FIGURE 3**

**STEROID HORMONE DOSE RESPONSE EFFECTS WITH  
 MTW9/PL2 CELLS IN 50% CDE - HORSE SERUM**

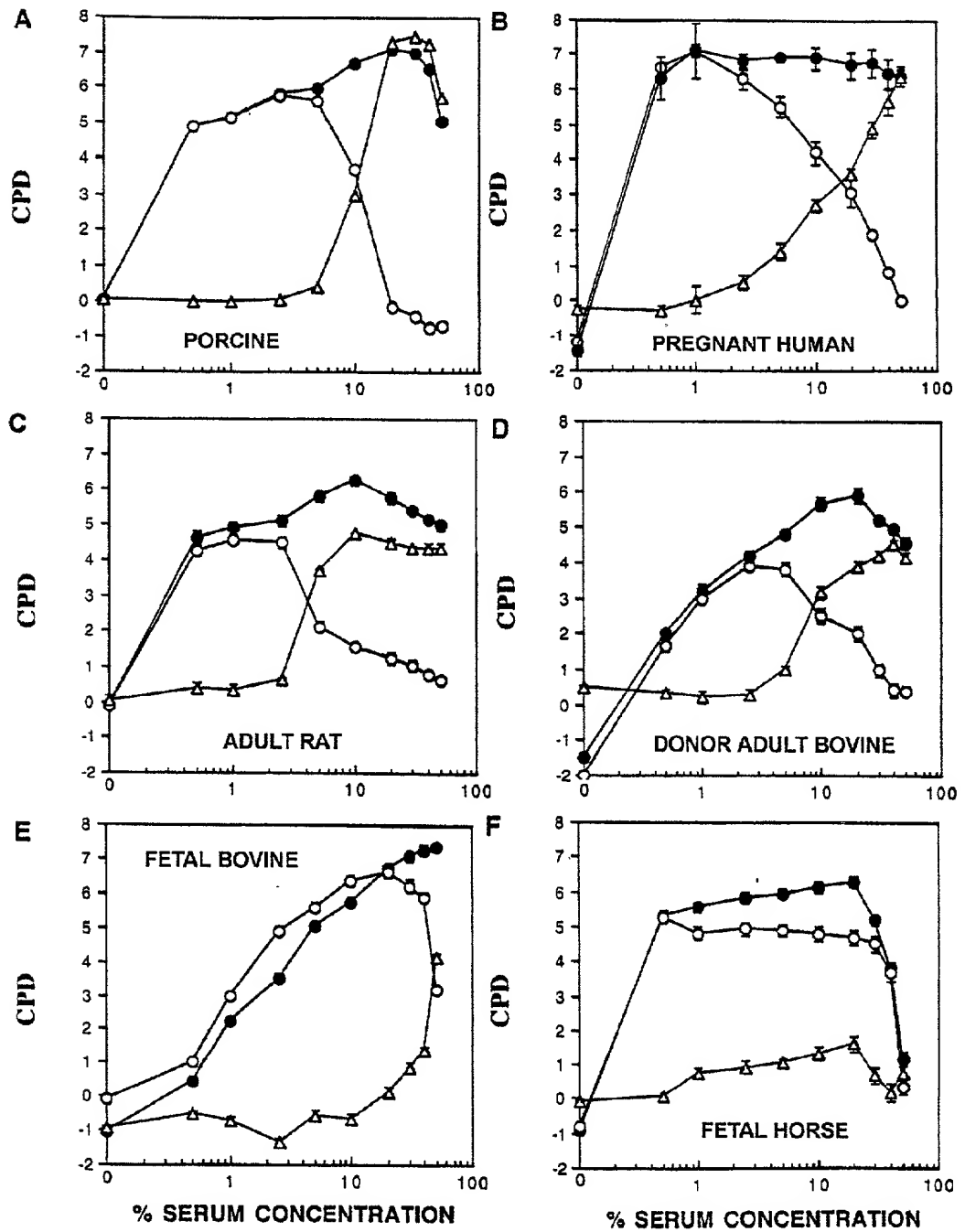


**LEGEND:**

Closed circles = E<sub>2</sub>  
 Open circles = E<sub>1</sub>  
 Closed squares = E<sub>3</sub>  
 Open squares = Progesterone  
 Closed triangles = DHT  
 Open triangles = T

FIGURE 4

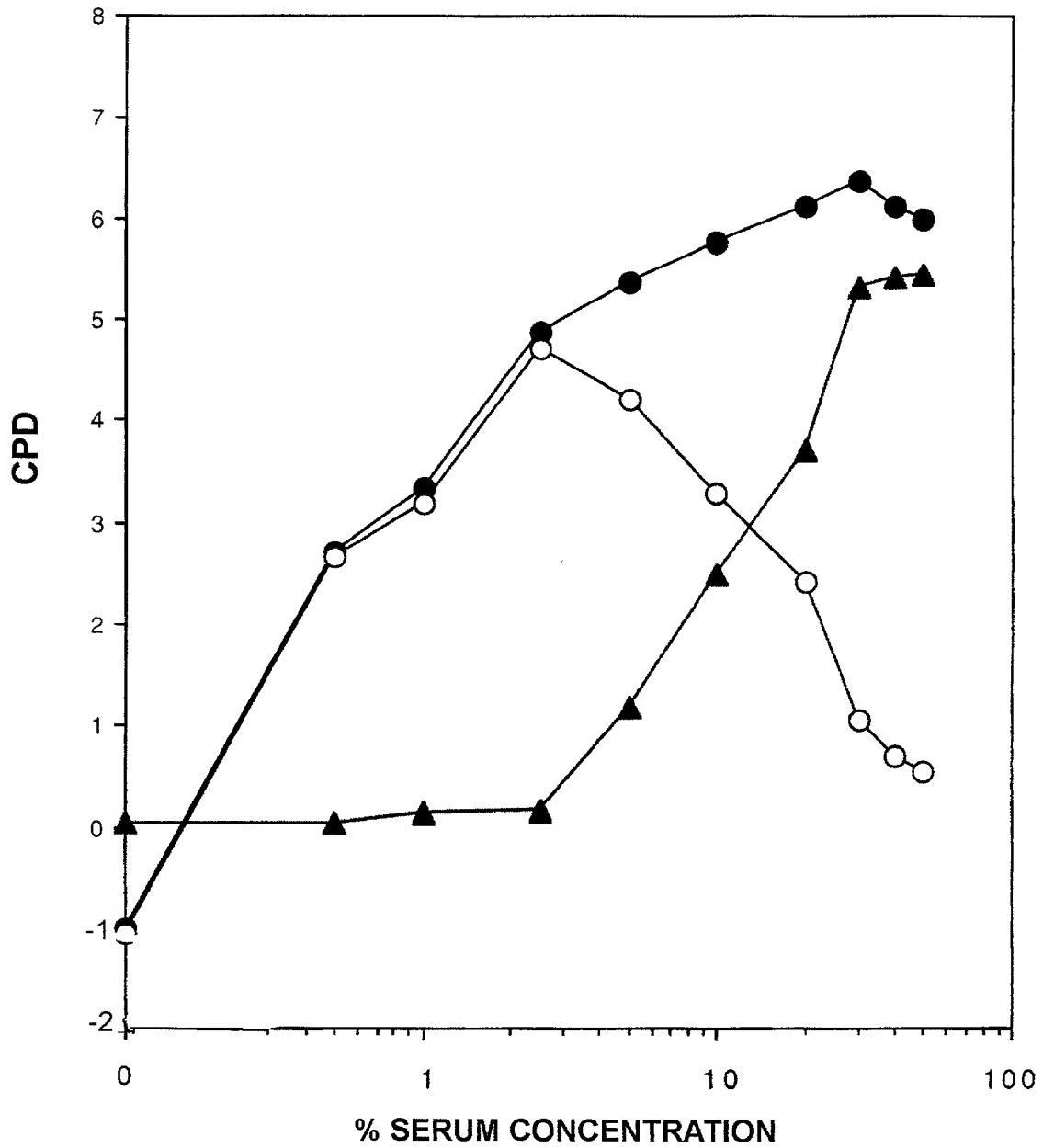
MTW9PL2 CELL GROWTH IN CDE SERUM  
 FROM DIFFERENT SPECIES



LEGEND: Open circles = -E<sub>2</sub>  
 Closed circles = +E<sub>2</sub>  
 Open triangles = Estrogenic effect

**FIGURE 5**

**CDE HORSE SERUM TITRATION  
GH4C1 CELLS**

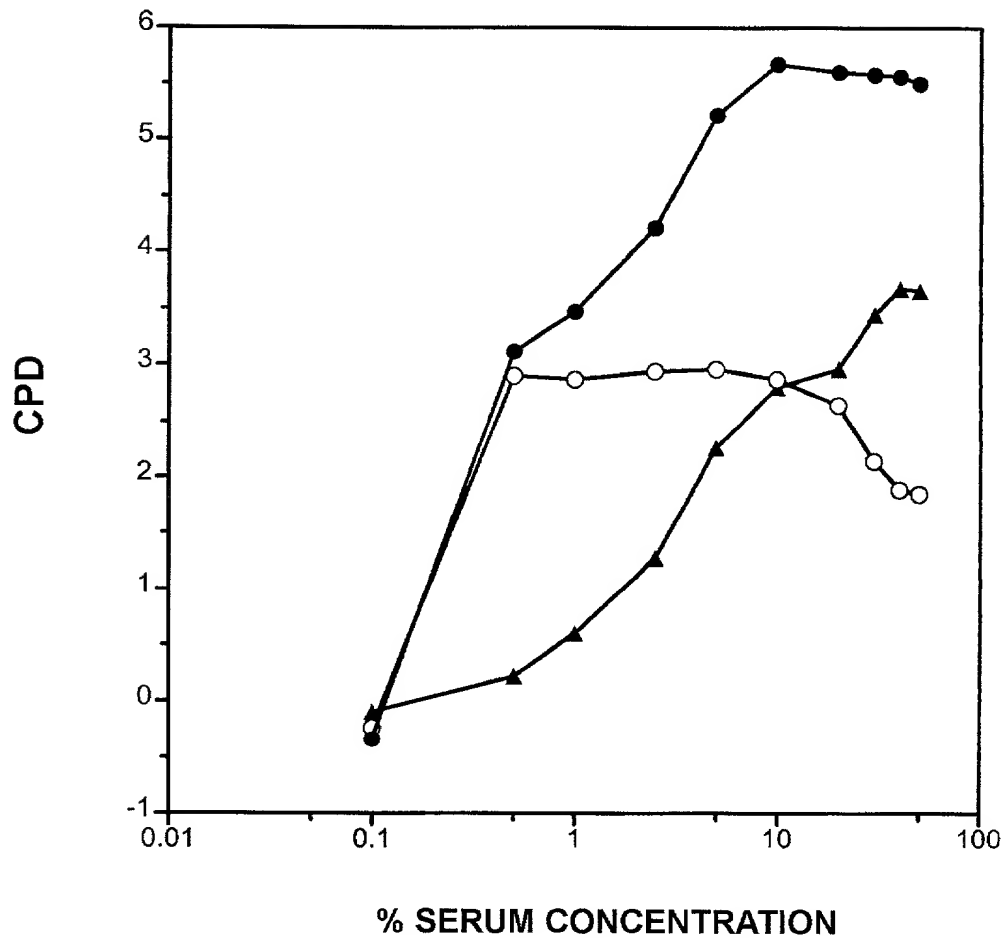


**LEGEND:**

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▲— = Estrogenic effect

**FIGURE 6**

**ZR-75-1 CELLS IN CDE - HORSE SERUM  $\pm$  10 nM  $E_2$**

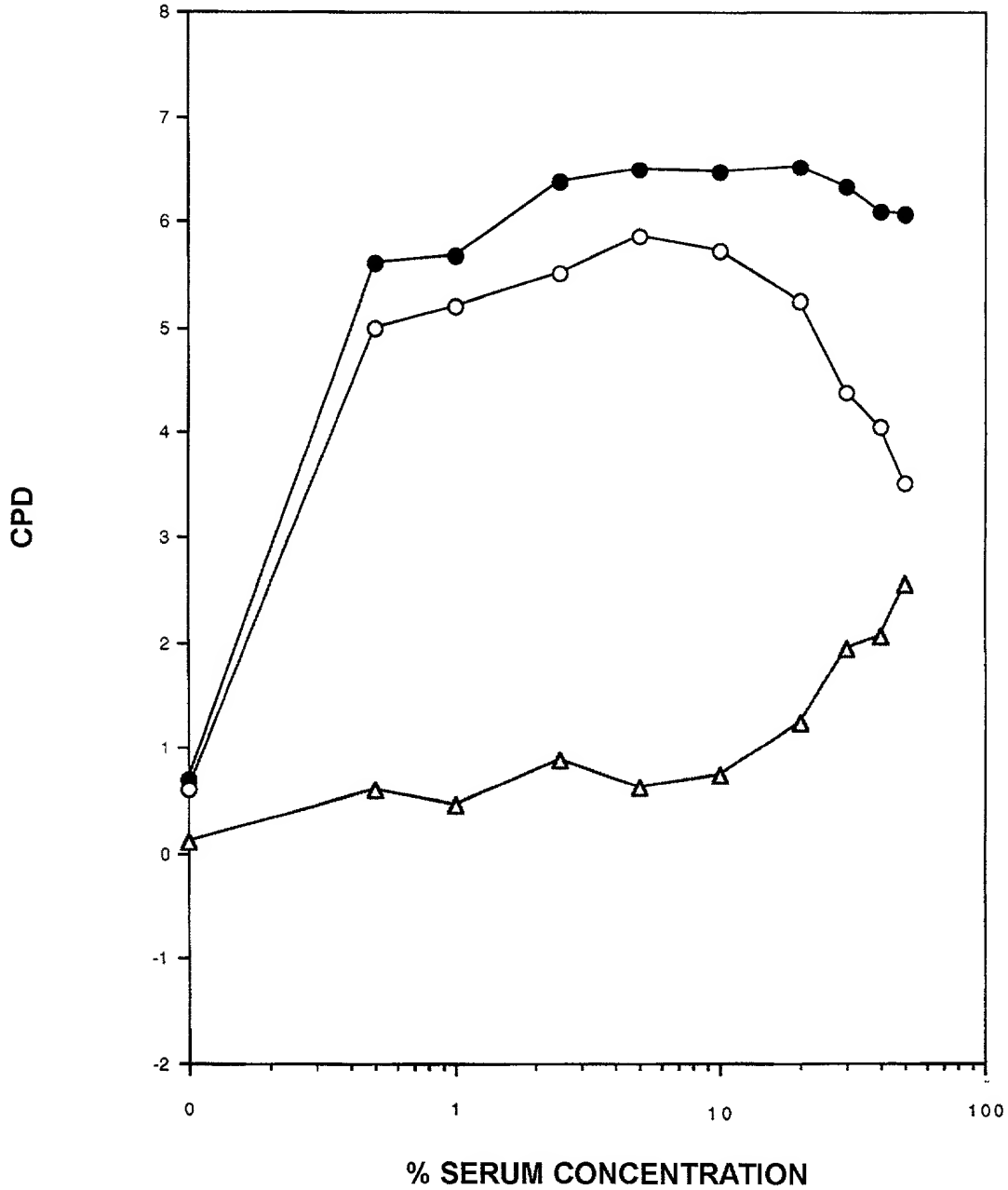


**LEGEND:**

Closed circles = +E<sub>2</sub>  
Open circles = -E<sub>2</sub>  
Closed triangles = Estrogenic effect

**FIGURE 7**

**MCF7A CELL GROWTH IN CDE - HORSE SERUM  $\pm$  E<sub>2</sub>**

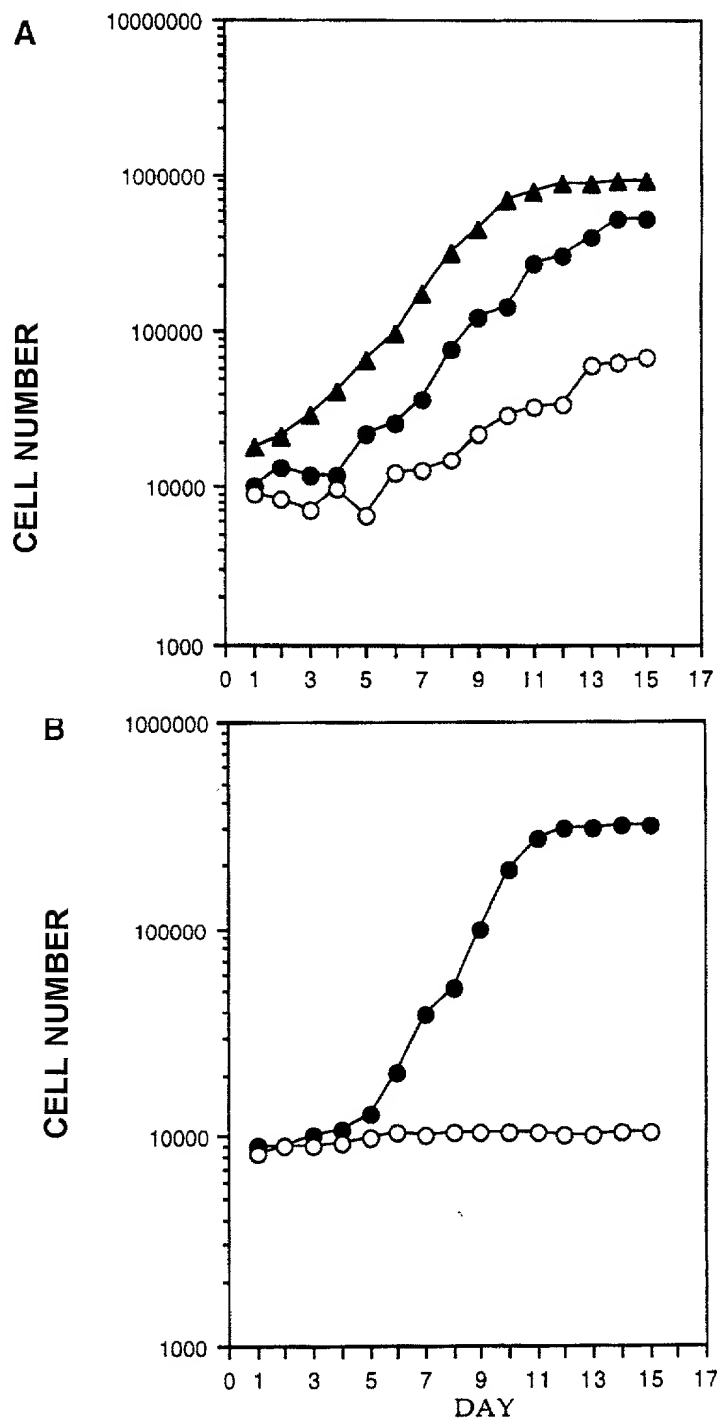


**LEGEND:**

Closed circles = +E<sub>2</sub>  
 Open circles = -E<sub>2</sub>  
 Closed triangles = Estrogenic effect

**FIGURE 8**

**GROWTH KINETICS OF T47D HUMAN BREAST CANCER  
 CELLS IN CDE - HORSE SERUM  $\pm 10$  nM  $E_2$**

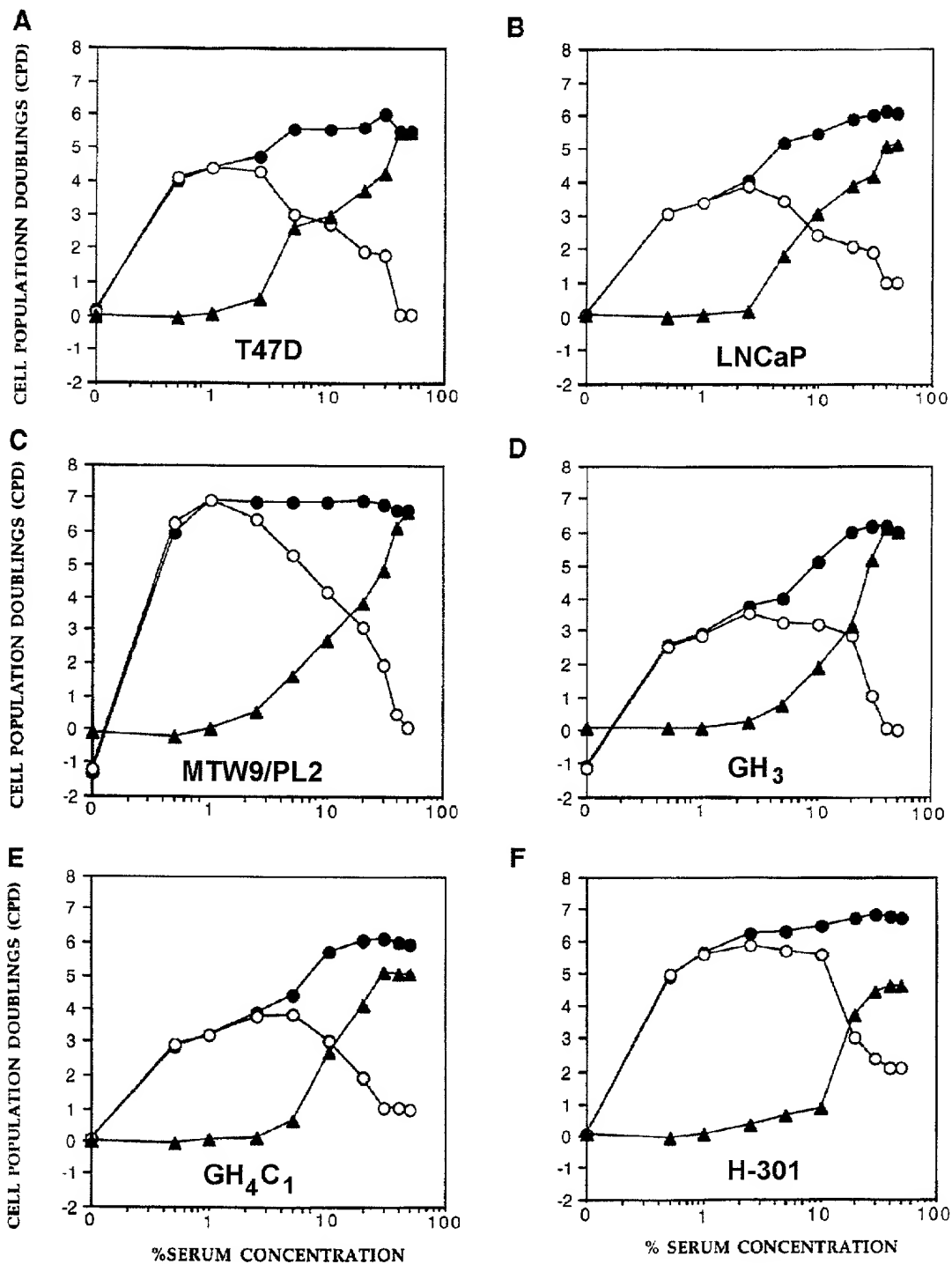


(A) The growth of the cells in medium with 20% (v/v) serum with 10 nM  $E_2$  (closed circles) and without the steroid (open circles). As comparison, growth is shown in medium containing 10% (v/v) FBS (triangles).

(B) T47D cell growth kinetics in medium with 50% (v/v) serum with  $E_2$  (closed circles) and without the steroid (open circles).

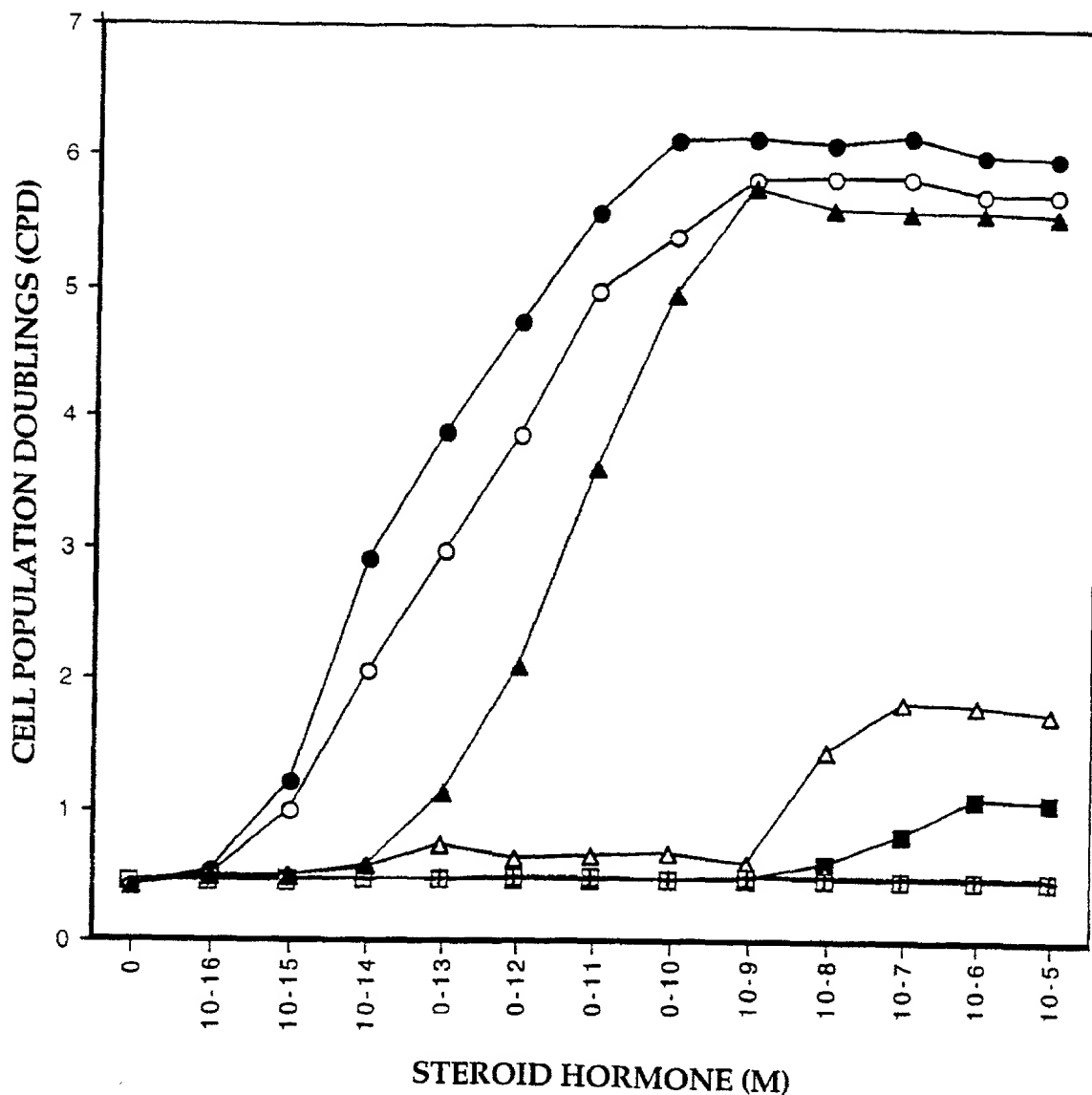
## FIGURE 9

### GROWTH OF HUMAN & RODENT CELL LINES IN 50% CDE - HORSE SERUM $\pm E_2$ (10 nM)



**FIGURE 10**

**DOSE RESPONSE OF STEROID HORMONES  
 WITH T47D CELLS IN 50% CDE - HORSE SERUM**



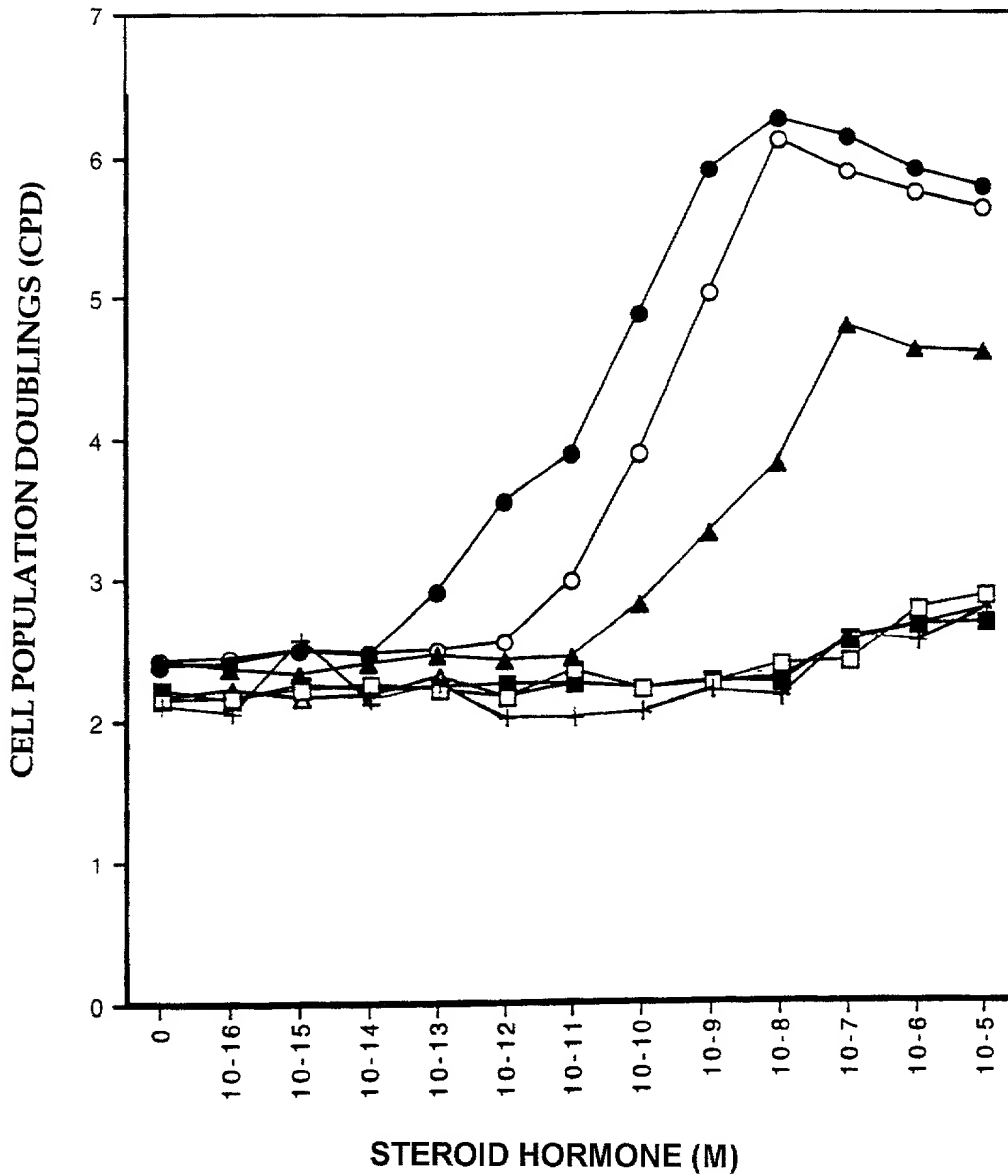
**LEGEND:**

Growth after 14 days is shown in response to:

- Closed circles = E<sub>2</sub>
- Open circles = E<sub>1</sub>
- Closed triangles = E<sub>3</sub>
- Open triangles = DHT
- Closed squares = Testosterone
- Open squares = Progesterone
- Crosses = Cortisol

**FIGURE 11**

**DOSE RESPONSE OF STEROID HORMONES  
 WITH H-301 CELLS IN 50% CDE - HORSE SERUM**



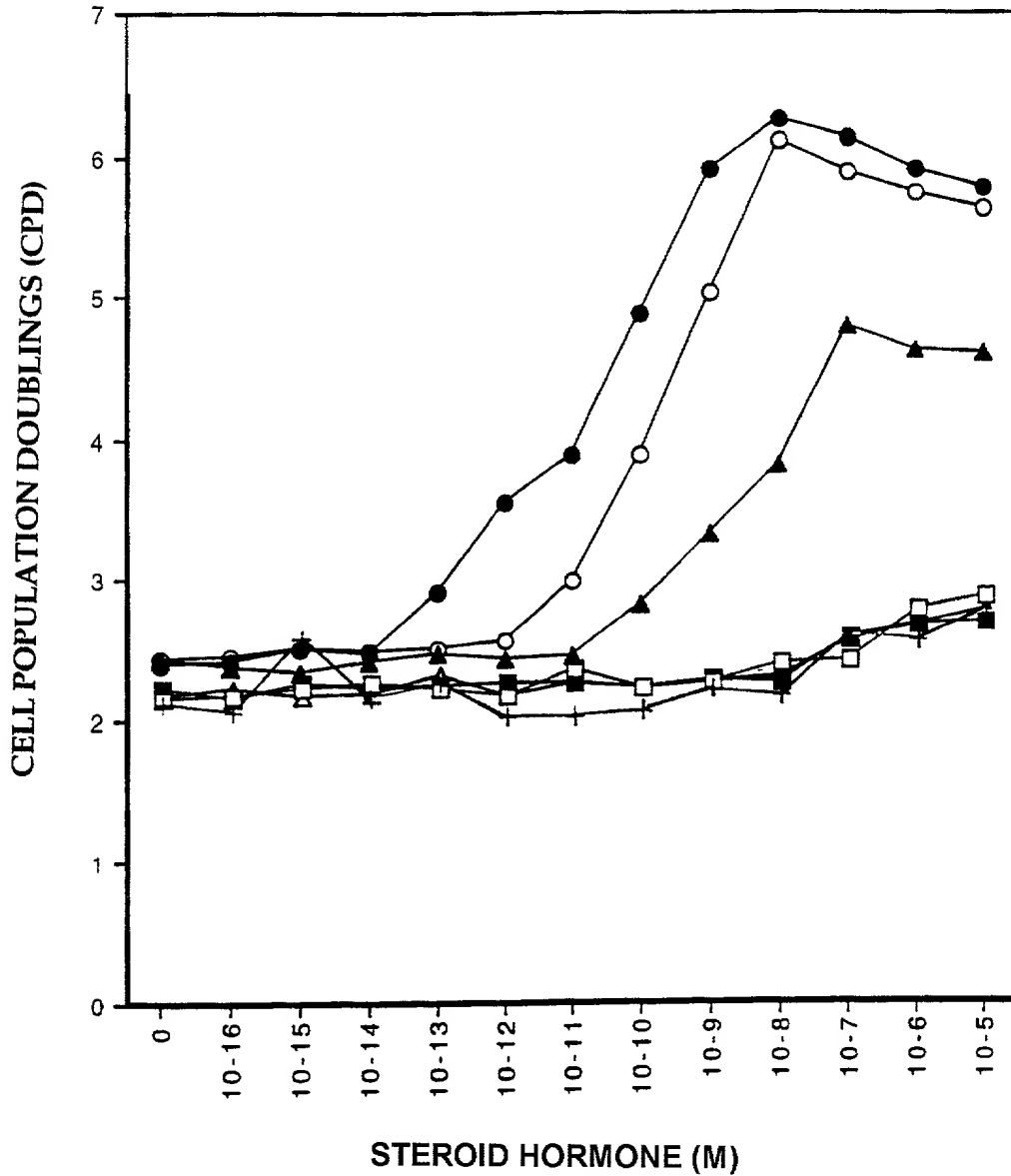
**LEGEND:**

Growth after 9 days is shown in response to:

- Closed circles = E<sub>2</sub>
- Open circles = E<sub>1</sub>
- Closed triangles = E<sub>3</sub>
- Open triangles = DHT
- Closed squares = Testosterone
- Open squares = Progesterone
- Crosses = Cortisol

**FIGURE 12**

**DOSE RESPONSE OF STEROID HORMONES  
 WITH H-301 CELLS IN 50% CDE - HORSE SERUM**



**LEGEND:**

Growth after 9 days is shown in response to:

Closed circles = E<sub>2</sub>

Open circles = E<sub>1</sub>

Closed triangles = E<sub>3</sub>

Open triangles = DHT

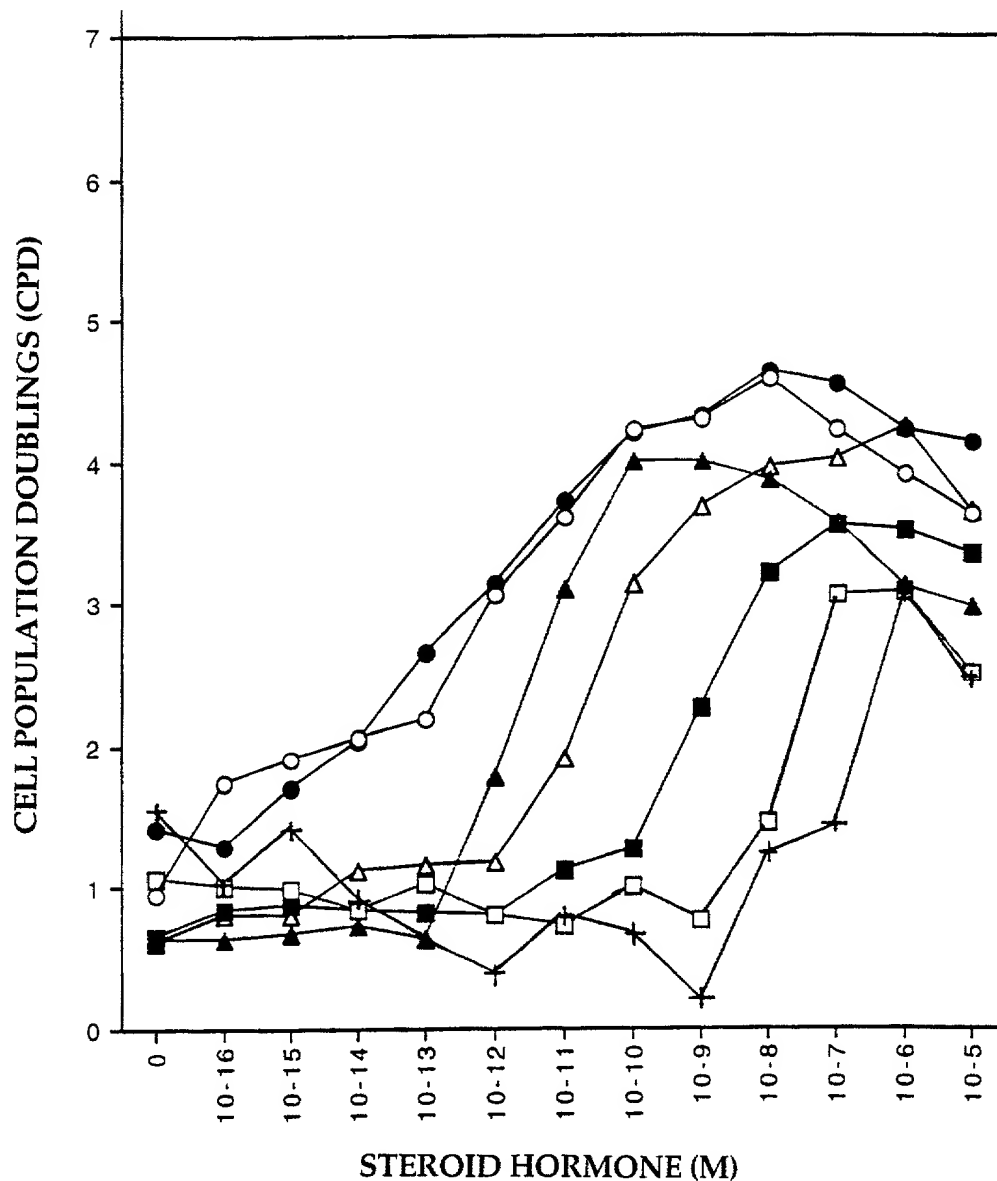
Closed squares = Testosterone

Open squares = Progesterone

Crosses = Cortisol

**FIGURE 13**

**DOSE RESPONSE OF STEROID HORMONES  
 WITH LNCaP CELLS IN 50% CDE - HORSE SERUM**



**LEGEND:**

Growth after 14 days is shown in response to:

- Closed circles = E<sub>2</sub>
- Open triangles = E<sub>1</sub>
- Open squares = E<sub>3</sub>
- Open circles = DHT
- Closed triangles = Testosterone
- Closed squares = Progesterone
- Crosses = Cortisol

**FIGURE 14**

**T<sub>3</sub> TITRATION OF GH<sub>3</sub> CELLS GROWN  
IN SERUM - FREE MEDIUM (PCM)**

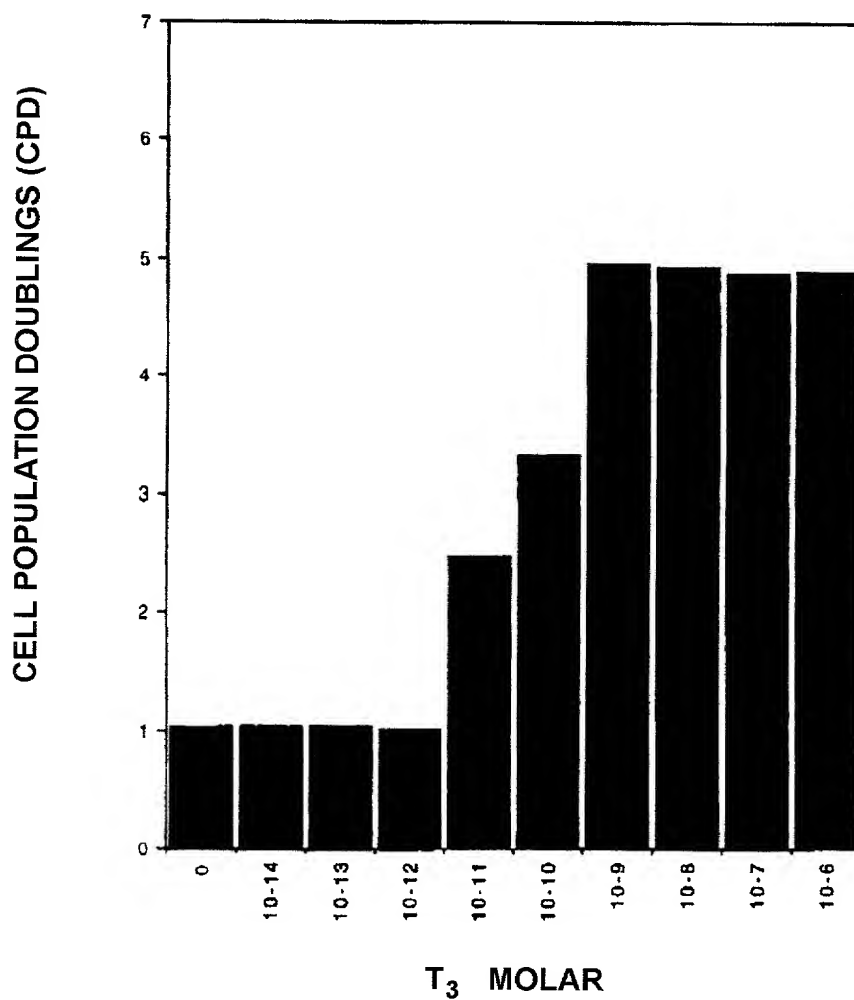
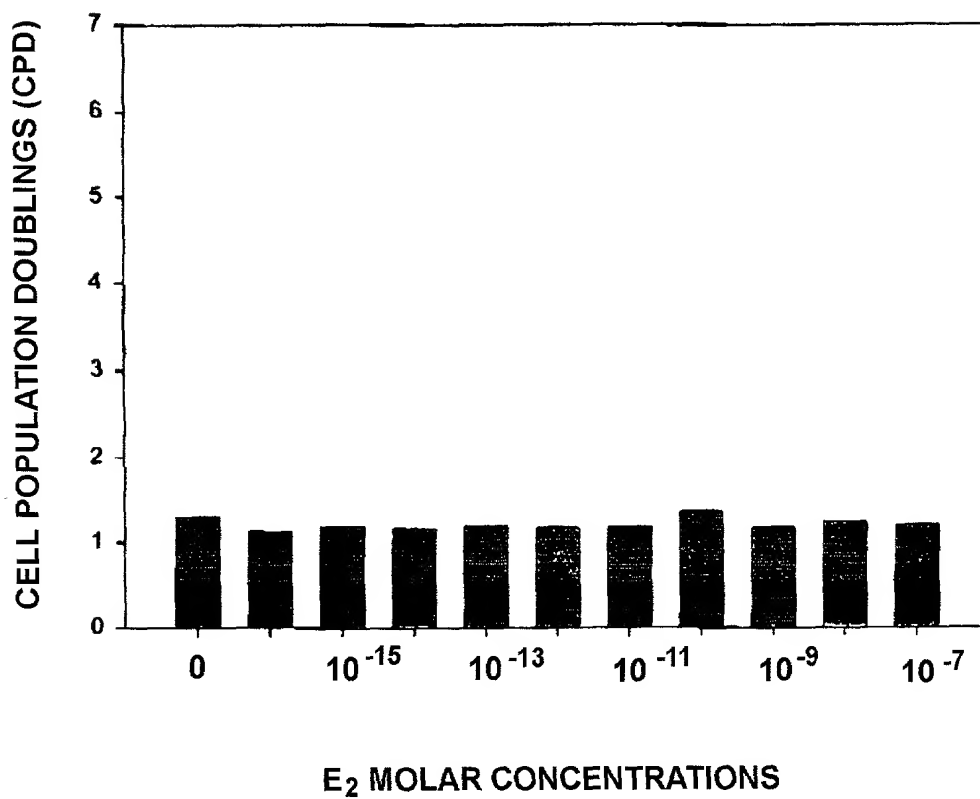


FIGURE 15

**E<sub>2</sub> TITRATION OF GH<sub>3</sub> CELLS GROWN IN  
SERUM-FREE MEDIUM MINUS T<sub>3</sub>**



**FIGURE 16**

**EFFECT OF  $T_3$  ON GH CELL LINES:  
GROWTH IN 2.5% CDE - HORSE SERUM WITH NO  $E_2$**

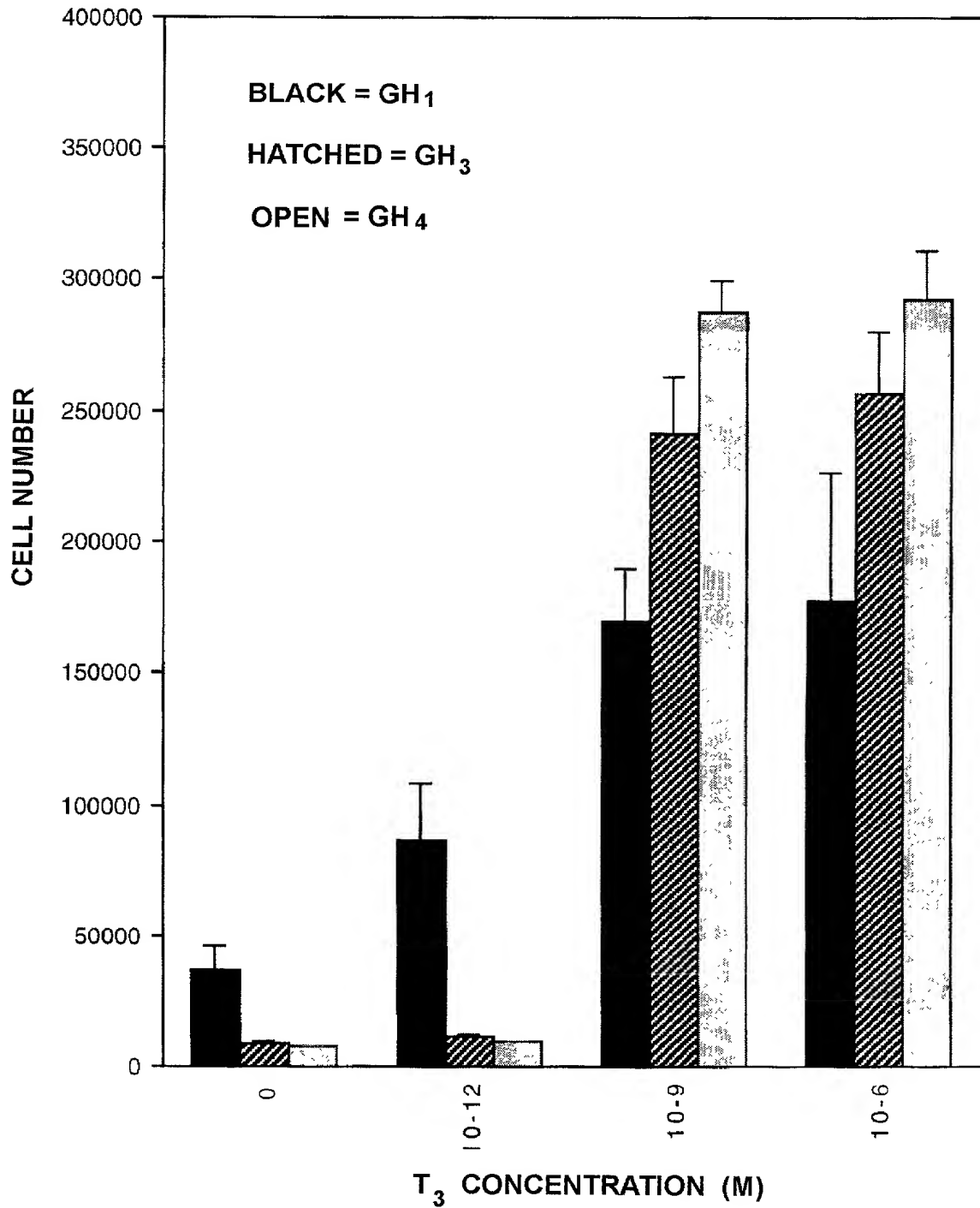
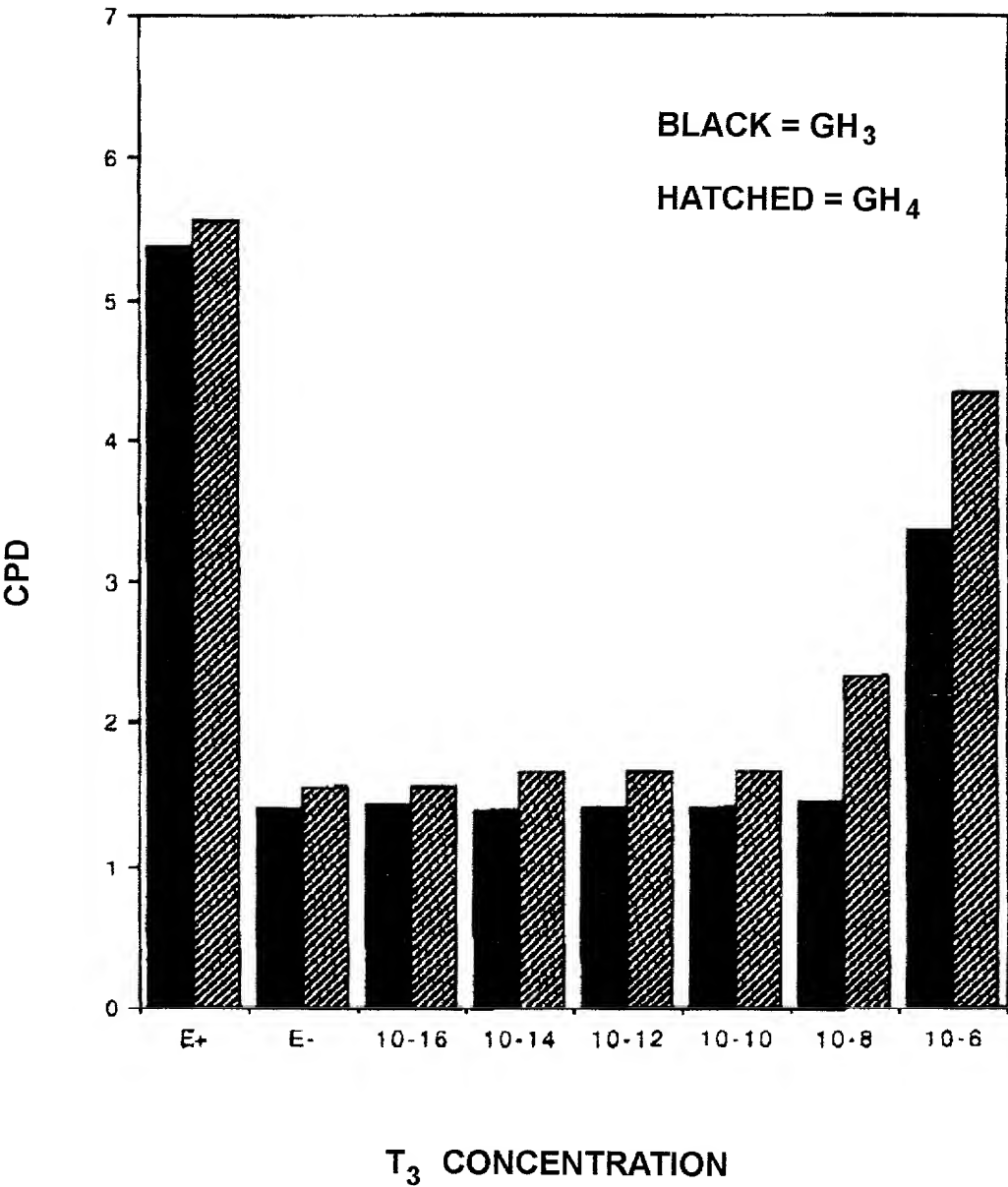


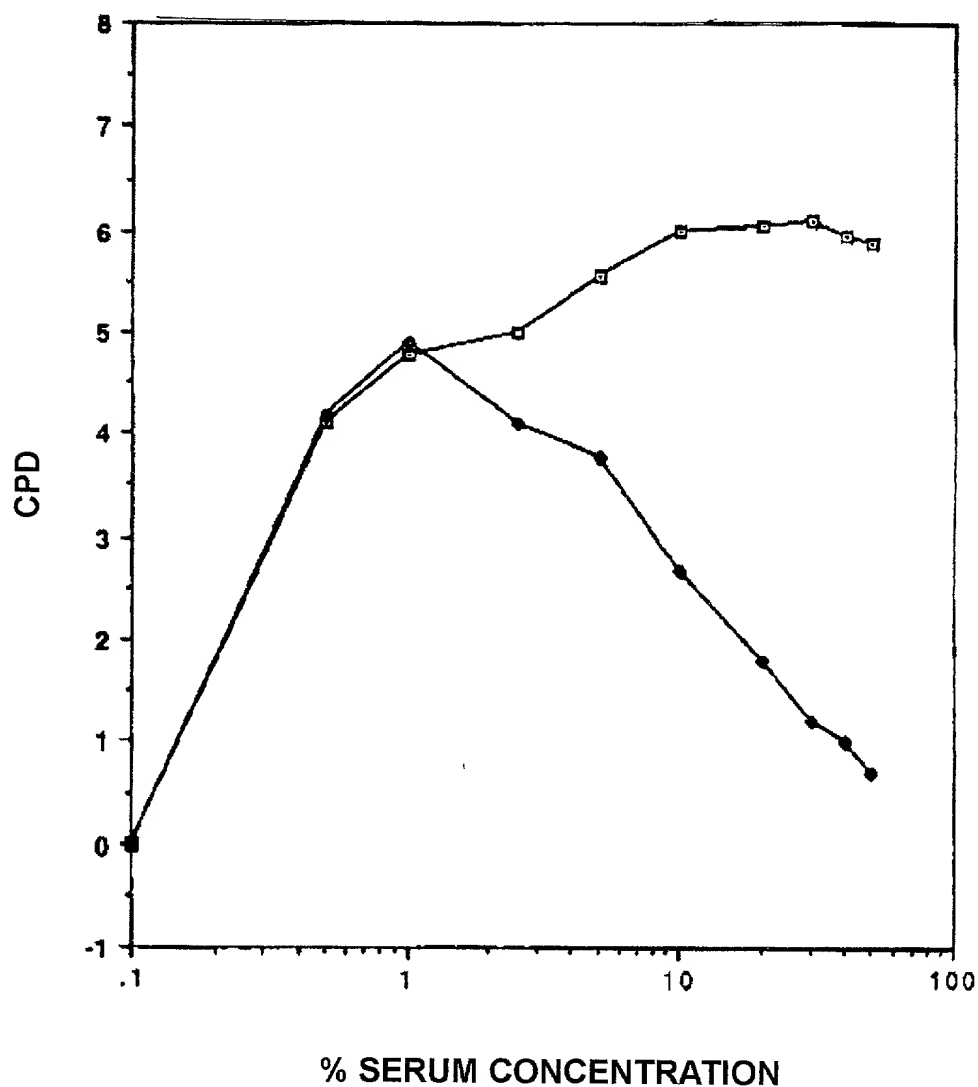
FIGURE 17

EFFECT OF T<sub>3</sub> ON PITUITARY CELL LINES  
INCUBATED IN 50% CDE - HORSE SERUM



**FIGURE 18**

**EFFECT OF XAD-4 RESIN TREATED HORSE SERUM  
ON MTW9/PL2 CELL GROWTH  $\pm E_2$**



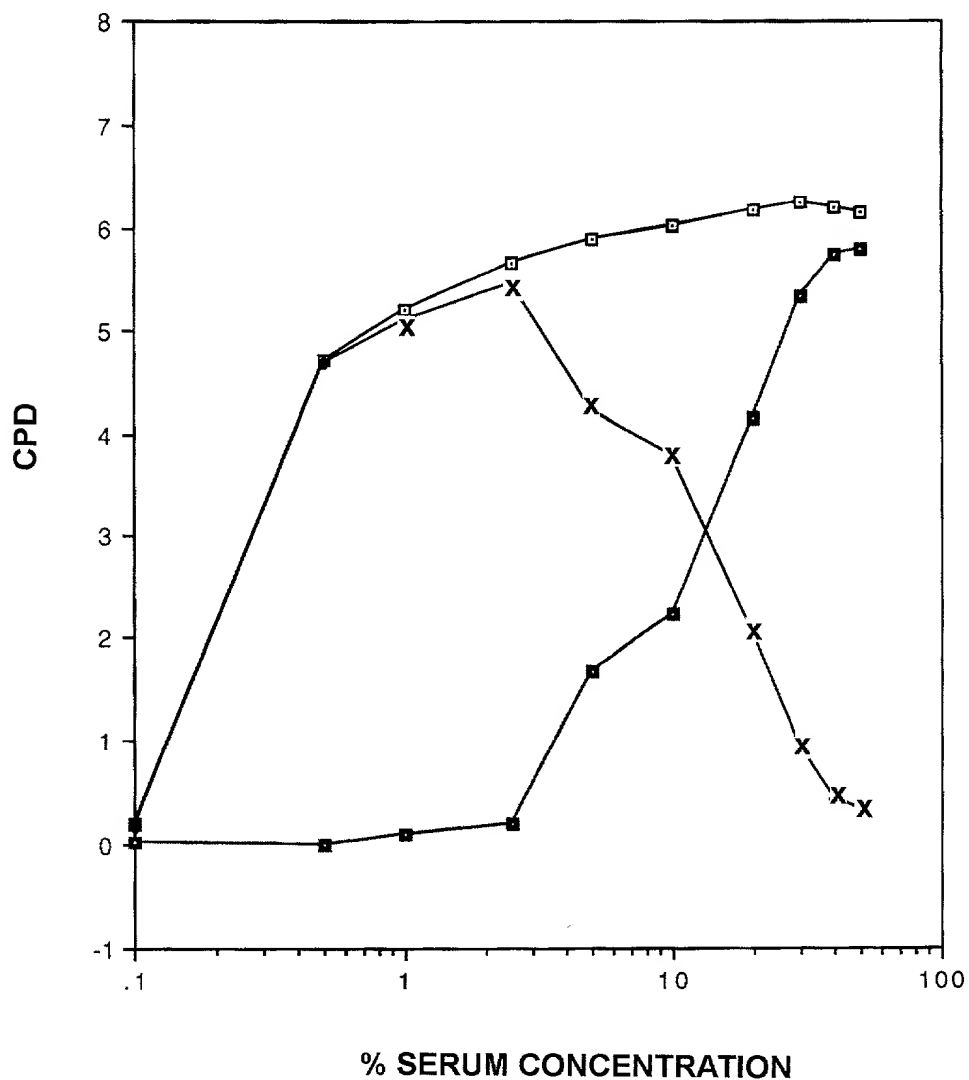
**LEGEND:**

Open squares = +  $E_2$

Closed squares = -  $E_2$

**FIGURE 19**

**EFFECT OF XAD-4 RESIN TREATED HORSE SERUM  
ON T47D CELL GROWTH  $\pm E_2$**



**LEGEND:**

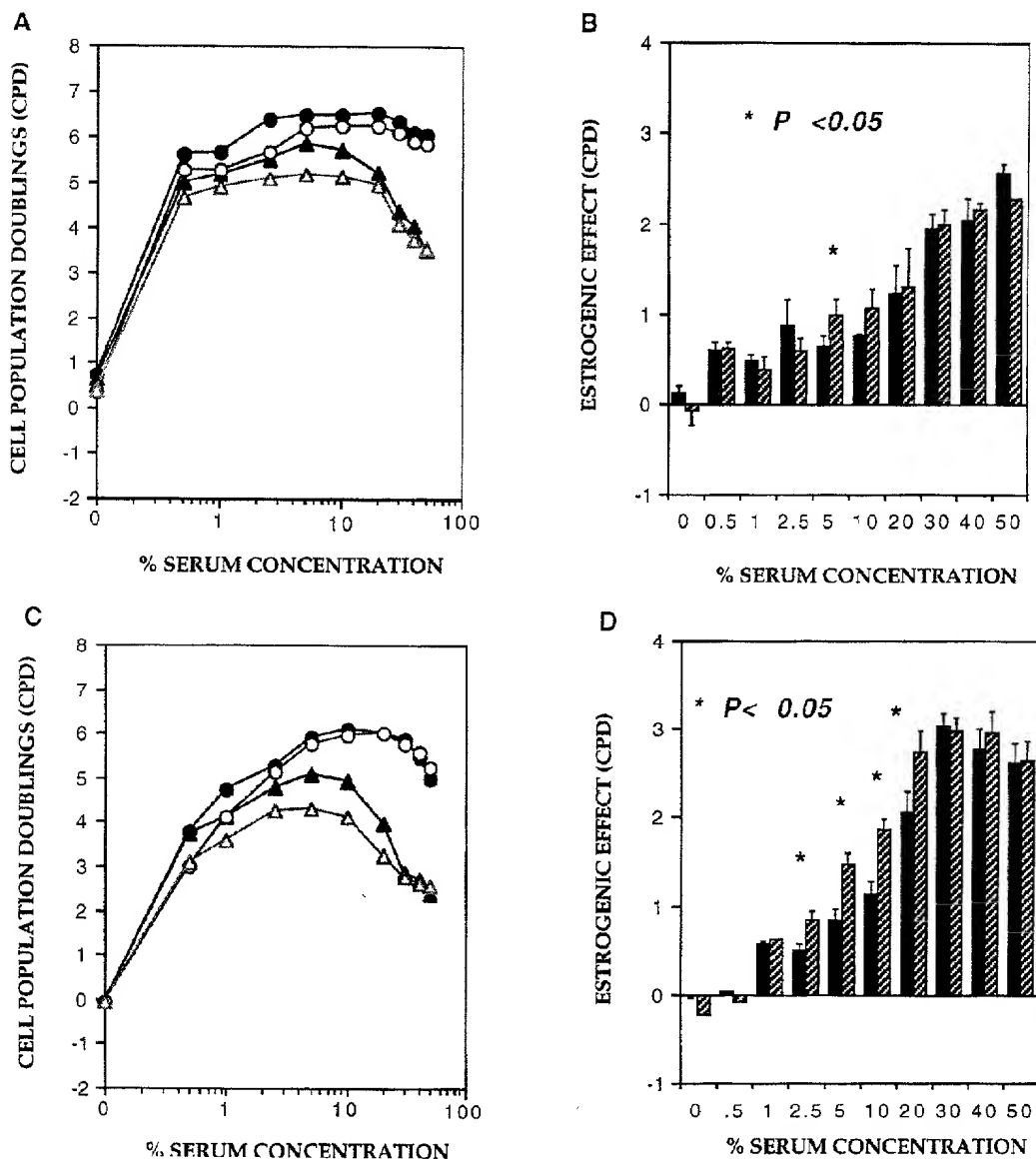
Open squares = + E<sub>2</sub>

XXX = - E<sub>2</sub>

Closed squares = Estrogenic effect

## FIGURE 20

### MCF-7 CELL GROWTH IN CDE - HORSE SERUM $\pm$ PHENOL RED AND $\pm$ E<sub>2</sub>

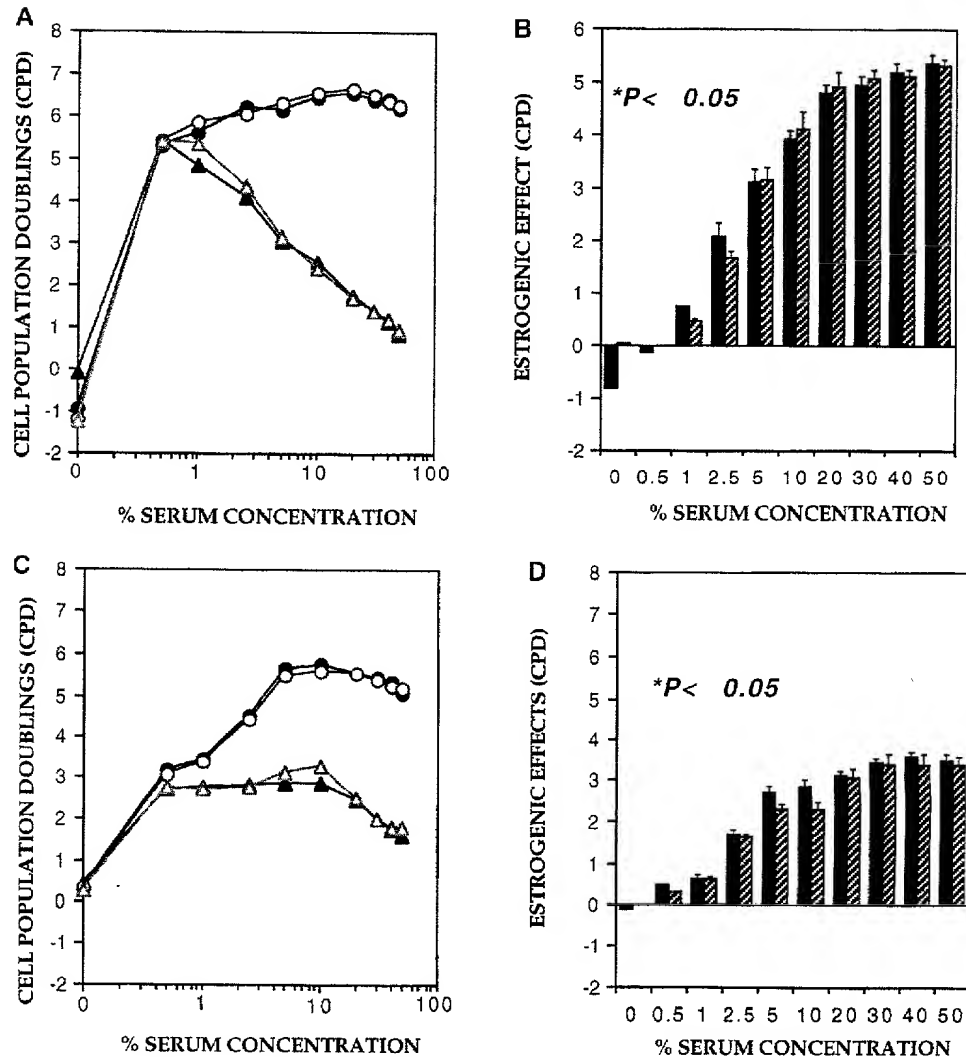


#### LEGEND:

- (A) MCF-7A cell growth in phenol red containing medium with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (closed triangles), and in phenol red-free medium with E<sub>2</sub> (open circles) and without E<sub>2</sub> (open triangles).  
 (B) Estrogenic effects with MCF-7A cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E<sub>2</sub> minus the CPD in medium without added E<sub>2</sub>.  
 (C) MCF-7K cell growth in phenol red medium with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (closed triangles), and in phenol red-free medium with E<sub>2</sub> (open circles) and without E<sub>2</sub> (open triangles).  
 (D) Estrogenic effects with MCF-7K cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

**FIGURE 21**

**T47D AND ZR-75-1 CELL GROWTH  
 IN CDE-HS  $\pm$  PHENOL RED AND  $\pm$  E<sub>2</sub>**

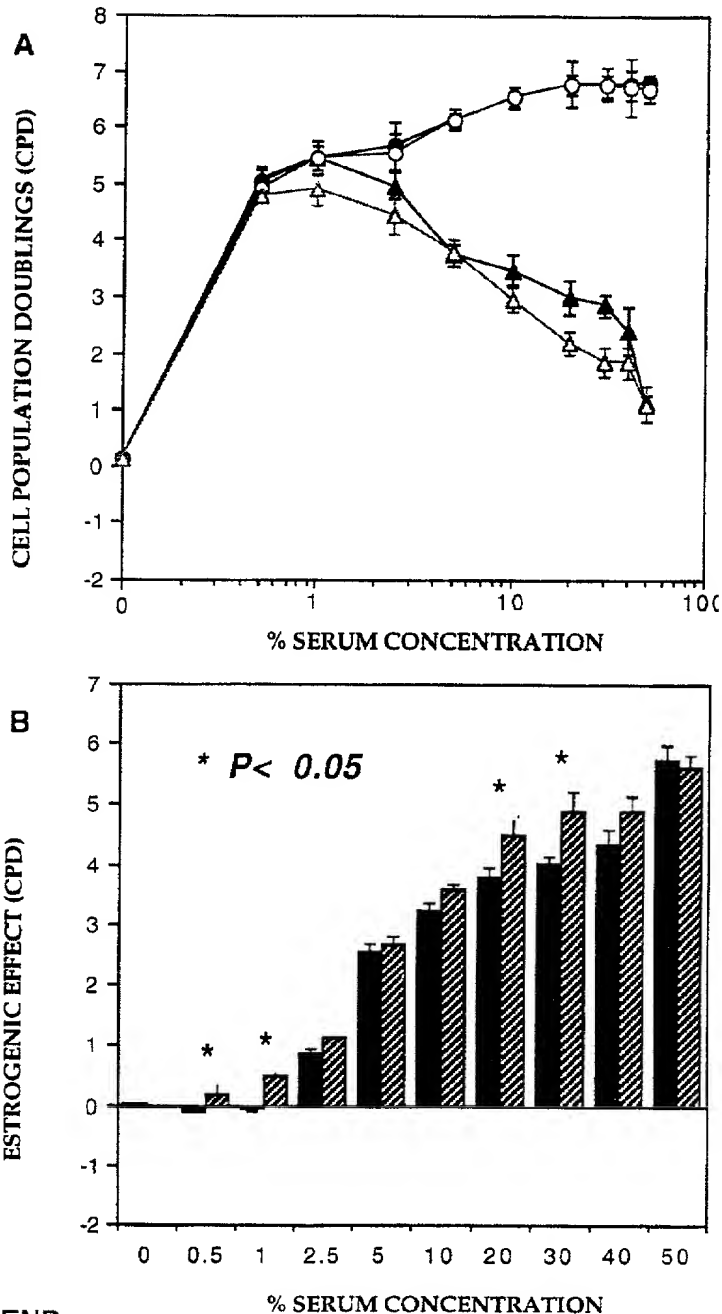


**LEGEND:**

- (A) T47D cell growth in phenol red containing medium with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (closed triangles), and in phenol red-free medium with E<sub>2</sub> (open circles) and without E<sub>2</sub> (open triangles).  
 (B) Estrogenic effects with T47D cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E<sub>2</sub> minus the CPD in medium without added E<sub>2</sub>.  
 (C) ZR-75-1 cell growth in phenol red medium with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (closed triangles), and in phenol red-free medium with E<sub>2</sub> (open circles) and without E<sub>2</sub> (open triangles).  
 (D) Estrogenic effects with ZR-75-1 cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 22

MTW9/PL2 CELL GROWTH IN CDE - HORSE SERUM  
 ± PHENOL RED AND ± E<sub>2</sub>



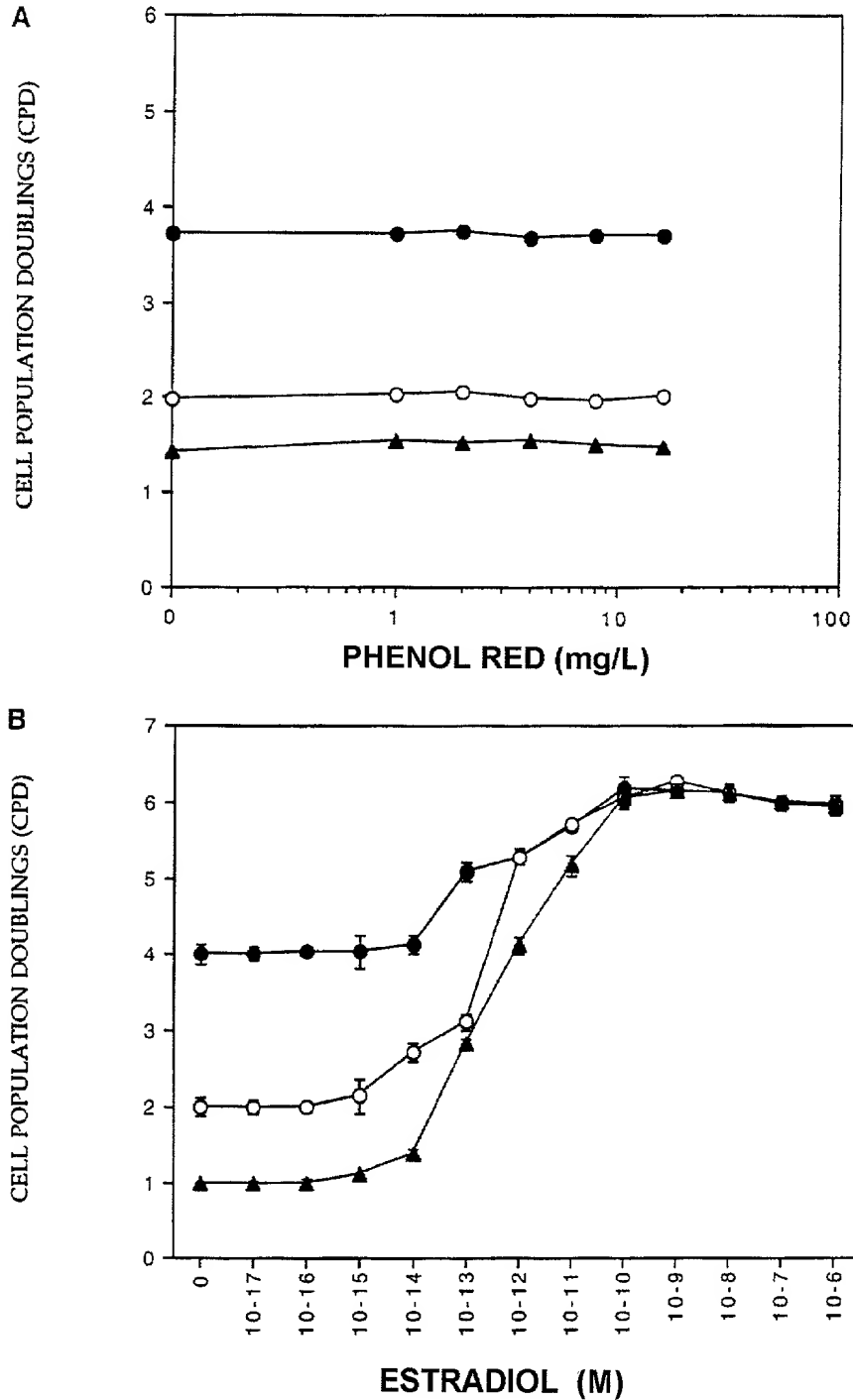
LEGEND:

(A) MTW9/PL2 growth in phenol red medium with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (closed triangles), and in phenol red-free medium with E<sub>2</sub> (open circles) and without E<sub>2</sub> (open triangles).

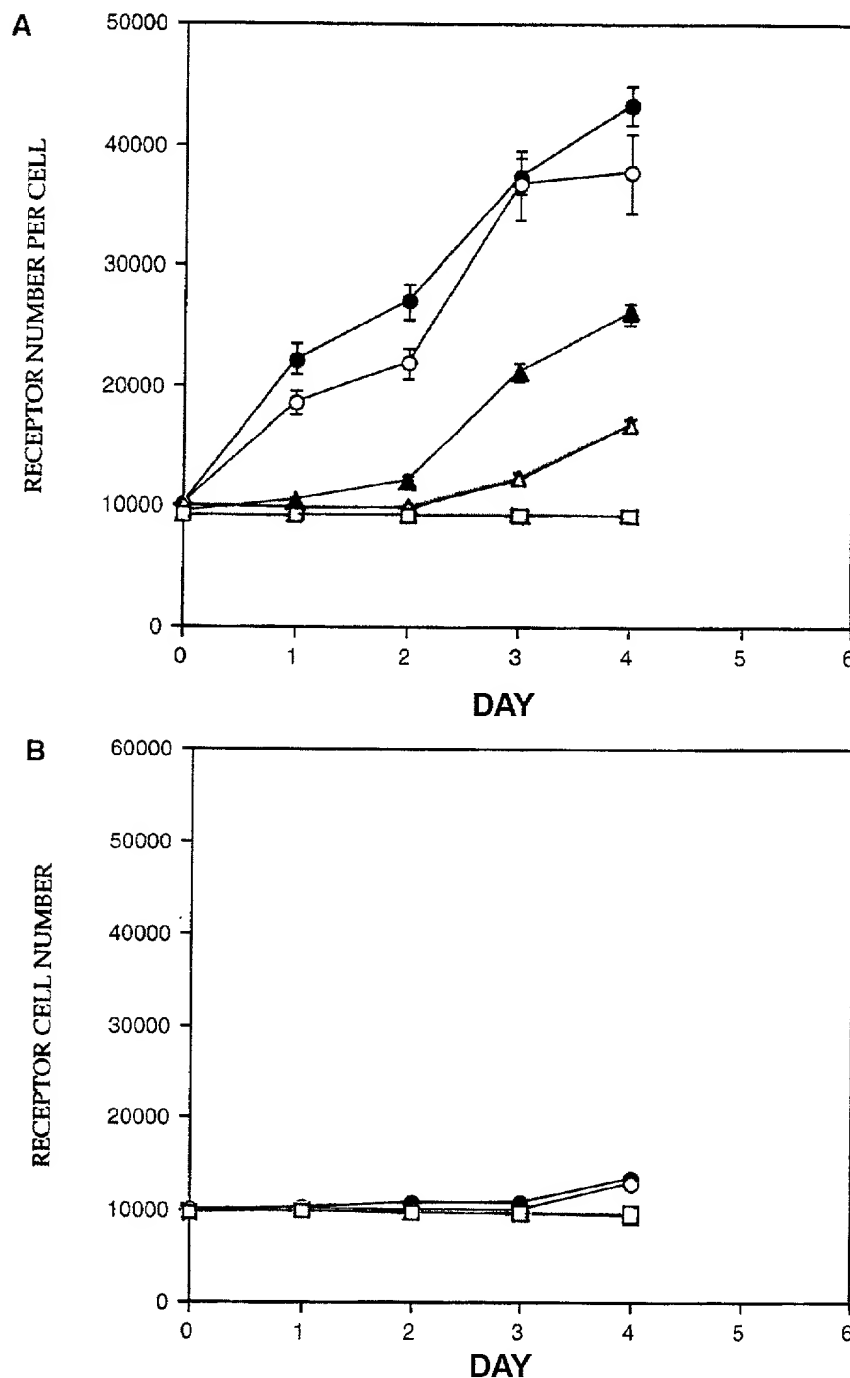
(B) Estrogenic effects with MTW9/PL2 cells in medium with phenol red (solid bars) and without (shaded bars) were calculated from (A).

**FIGURE 23**

**DOSE RESPONSE TO PHENOL RED AND E<sub>2</sub>  
 IN THREE CELL LINES**



**LEGEND:** The growth of the MCF-7A (closed circles), MTW9/PL2 (open circles) and T47D (closed triangles) cell lines was assessed at 14, 7, and 12 days.

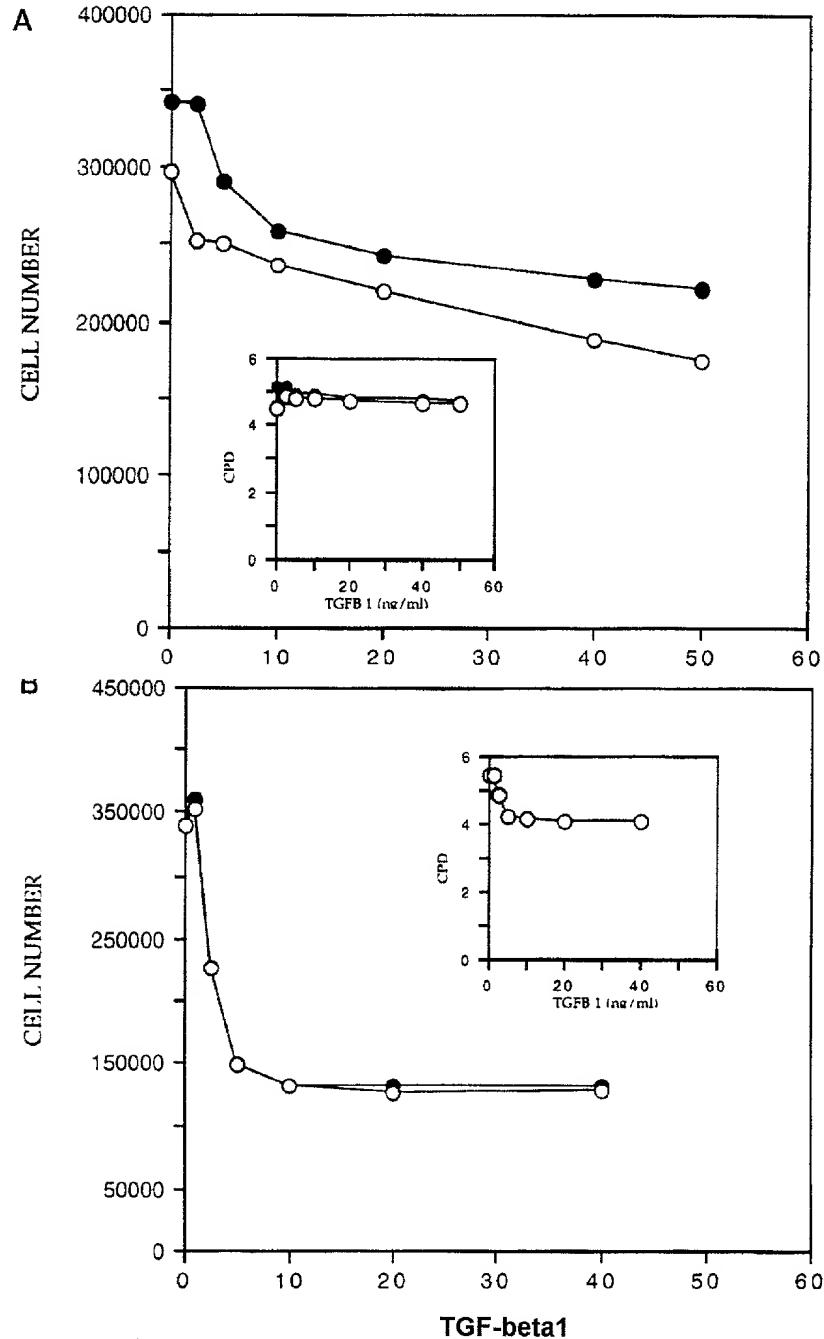
**FIGURE 24****PROGESTERONE RECEPTOR INDUCTION IN  
T47D CELLS BY PHENOL RED AND E<sub>2</sub>****LEGEND:**

(A) The effects of E<sub>2</sub> at 1.0 x 10<sup>-8</sup> M (closed circles), 1.0 x 10<sup>-10</sup> M (open circles), 1.0 x 10<sup>-12</sup> M (closed triangles), 1.0 x 10<sup>-14</sup> M (open triangles) and the control without added E<sub>2</sub> (open squares).

(B) The effects of phenol red at 16 mg/L (closed circles), 8 mg/L (open circles), 4 mg/L (closed triangles), 2 mg/L (open triangles), and the control without phenol red (open squares).

**FIGURE 25**

**EFFECT OF TGF-beta1 ON THE GROWTH OF BREAST/MAMMARY ORIGIN CELL LINES**



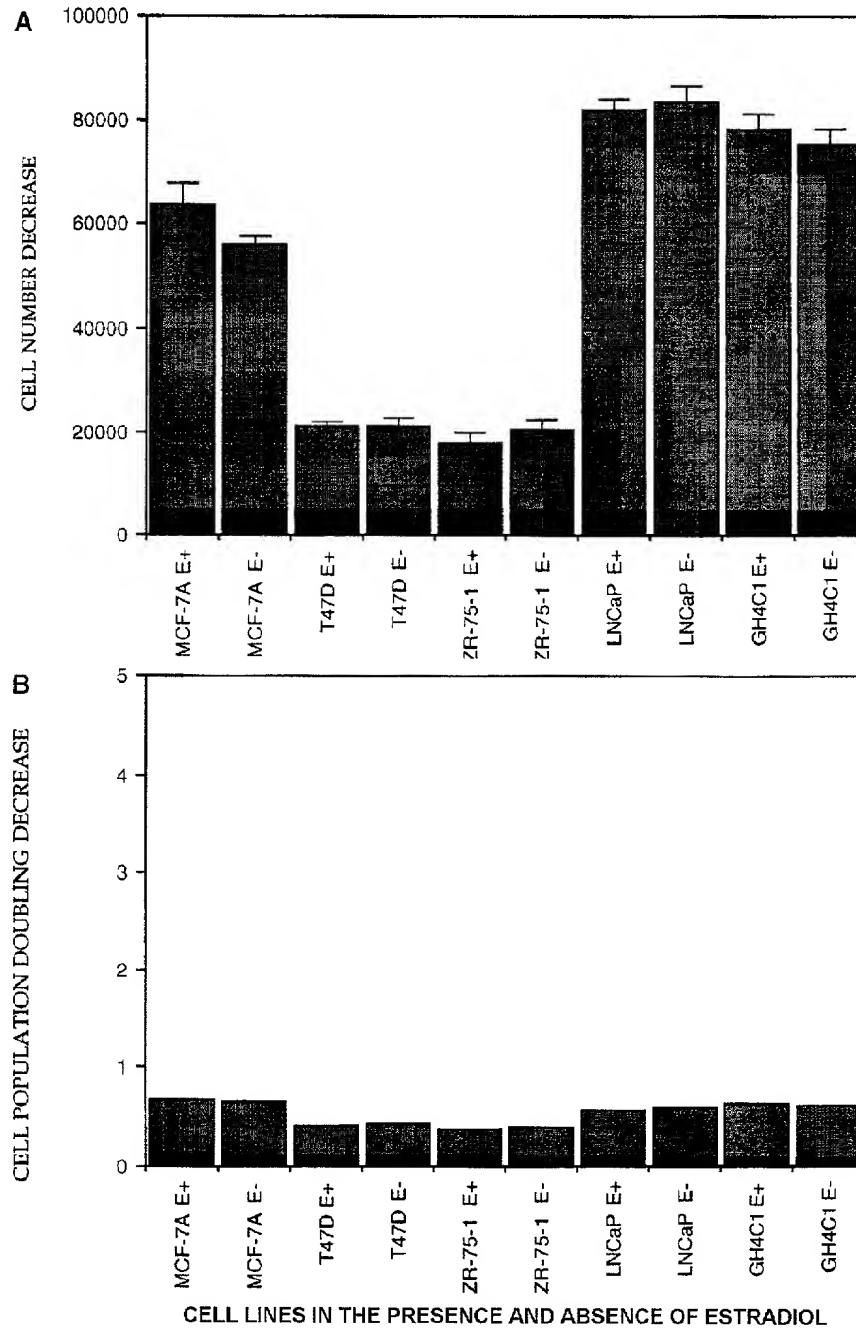
**LEGEND:**

(A) The effect of the transforming growth inhibitor on human breast MCF-7K cell growth as measured after 12 d either with 10 nM E<sub>2</sub> (closed circles) or without the hormone (open circles). The insert shows conversion of the cell number results to CPD.

(B) The same experiment with rat mammary MTW9/PL2 cells after 9 d growth.

## FIGURE 26

### EFFECT OF TGF-beta1 ON THE GROWTH OF CELL LINES FROM BOTH HUMAN AND RODENT TUMORS



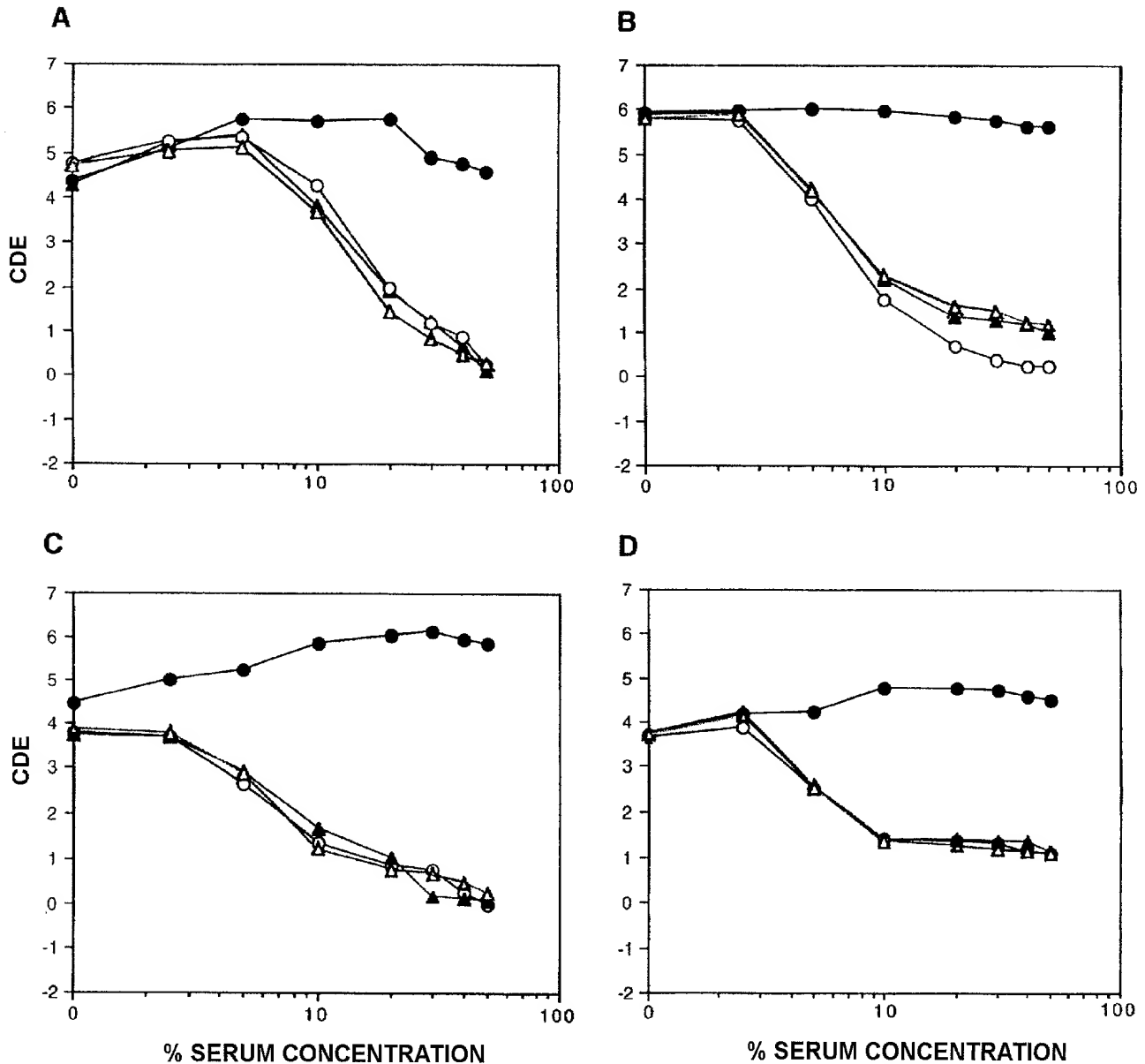
In these studies, TGF-beta1 was added at 40 ng/ml. Estradiol ( $\pm$  E) indicates either no added  $E_2$  or the steroid at 10 nM.

(A) The effect of TGF-beta1 on five cell lines after 10-14 d growth in medium  $\pm$   $E_2$ . The results are expressed as cell number decreases caused by TGF-beta1.

(B) The CPD decreases caused by TGF-beta1  $\pm$   $E_2$  with each of the cell lines shown in (A).

**FIGURE 27**

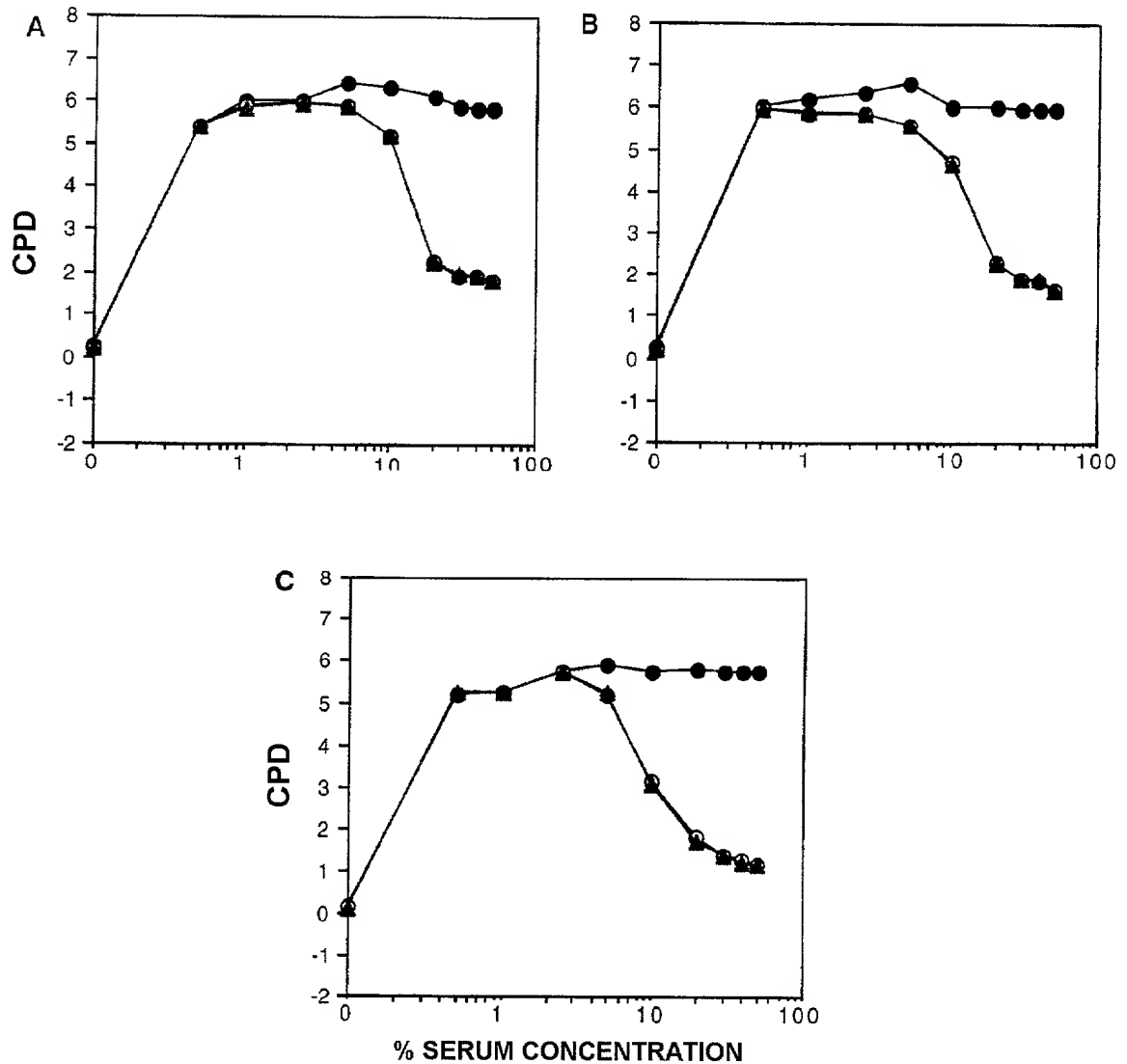
**EFFECT OF EGF AND TGF-alpha ON THE GROWTH  
 OF HUMAN BREAST CANCER CELLS**



The cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each line tested was grown in serum alone (open circles) and in serum plus 50 ng/ml EGF (open triangles), 50 ng/ml TGF-alpha (closed triangles), or 10 nM E<sub>2</sub> without exogenous growth factors (closed circles). (A) - (D) show the results with the MCF-7A, MCF-7K, T47D, and ZR-75-1 cell lines, respectively.

FIGURE 28

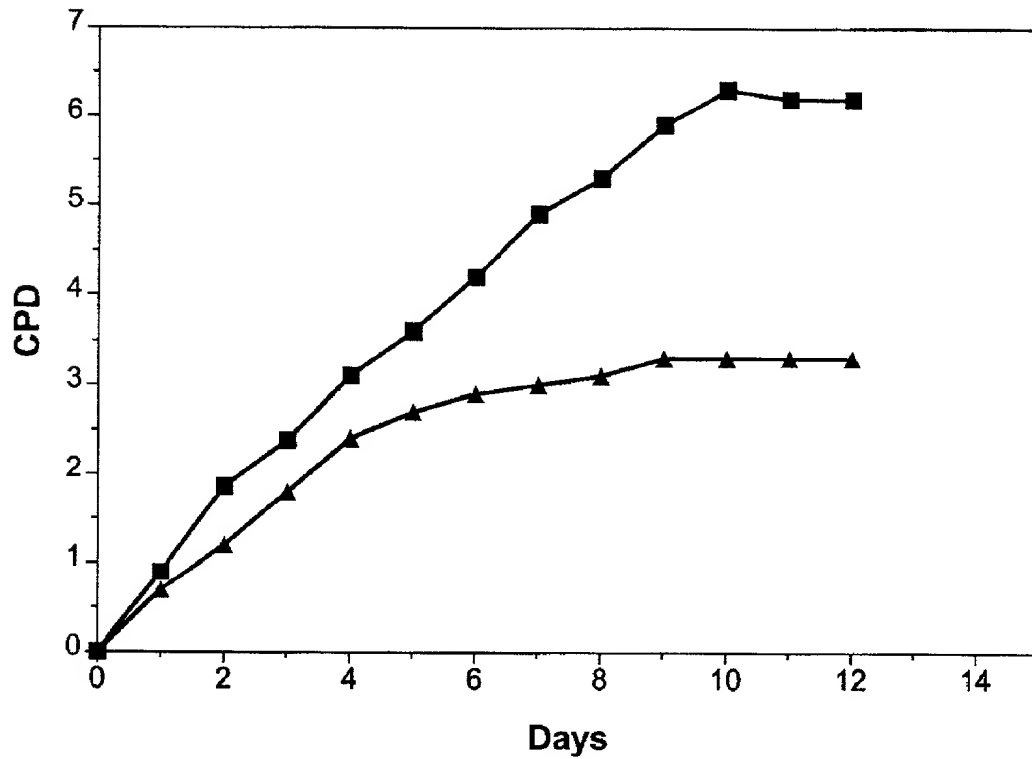
EFFECT OF IGF-I ON THE GROWTH  
 OF HUMAN BREAST CANCER CELLS



Breast cancer cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each cell line tested was grown in serum alone (open circles) and in serum plus 1.0 ug/ml IGF-I (triangles), or in serum with 10 nM E<sub>2</sub> without exogenous growth factors (closed circles). (A) - (C) show the results with the MCF-7K, MCF-7A and T47D cells, respectively. Assays were conducted for 12-14 d.

**FIGURE 29**

**T47D CELLS IN STANDARD D-MEM/F-12 MEDIUM  
VS "LOW FE" SERUM-FREE SERUM**

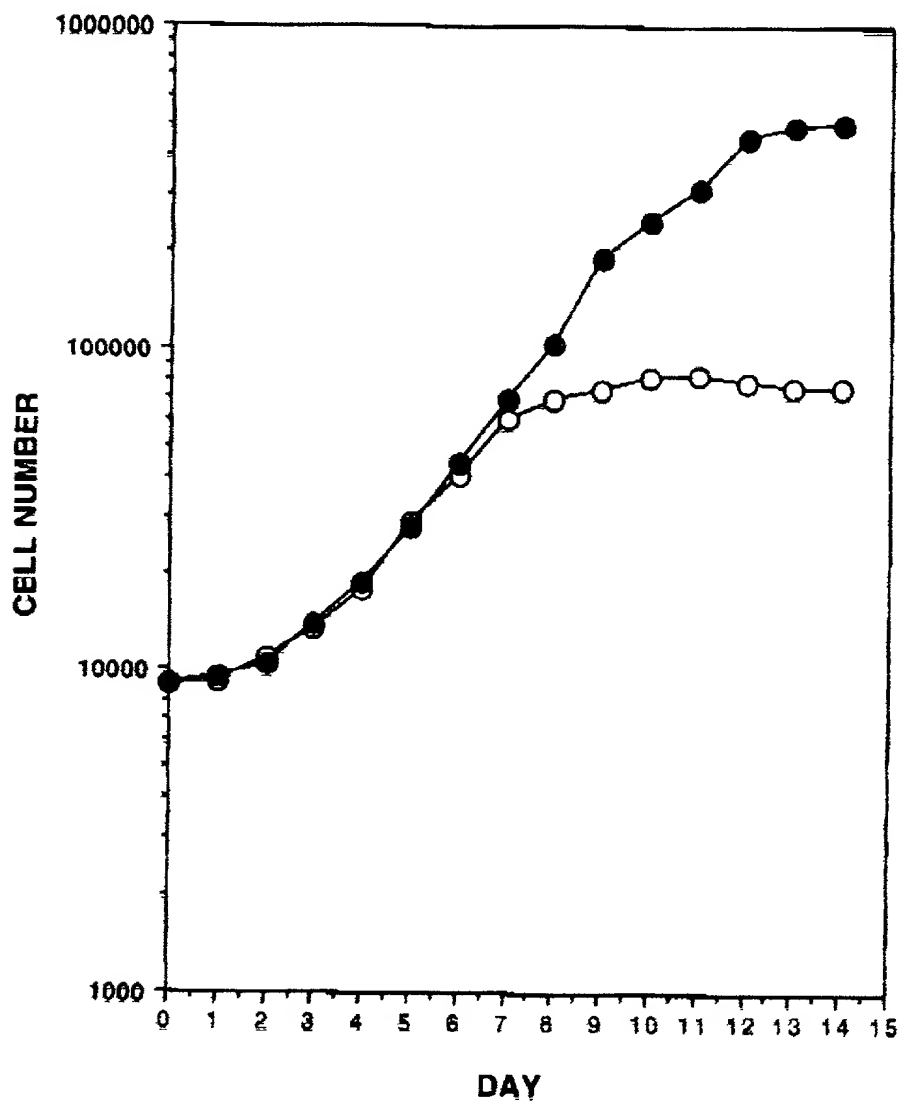


**LEGEND:**

- "STANDARD" MEDIUM
- ▲— "LOW-FE" MEDIUM

**FIGURE 30**

**LNCaP CELLS IN STANDARD D-MEM/F-12 MEDIUM  
VS "LOW-FE" SERUM-FREE MEDIUM**

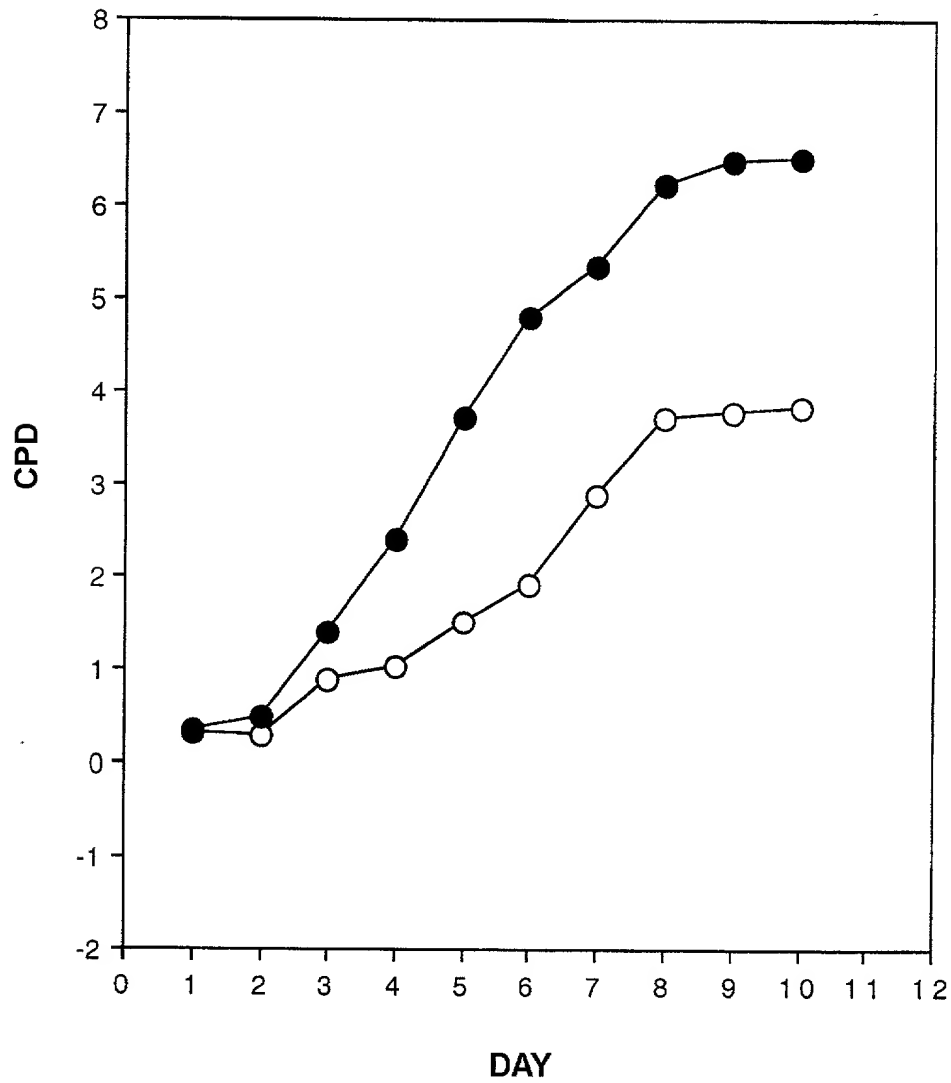


LEGEND:

- "STANDARD" MEDIUM
- "LOW-FE" MEDIUM

**FIGURE 31**

**MDCK CELLS IN STANDARD D-MEM/F-12 MEDIUM  
VS "LOW FE" SERUM-FREE MEDIUM**



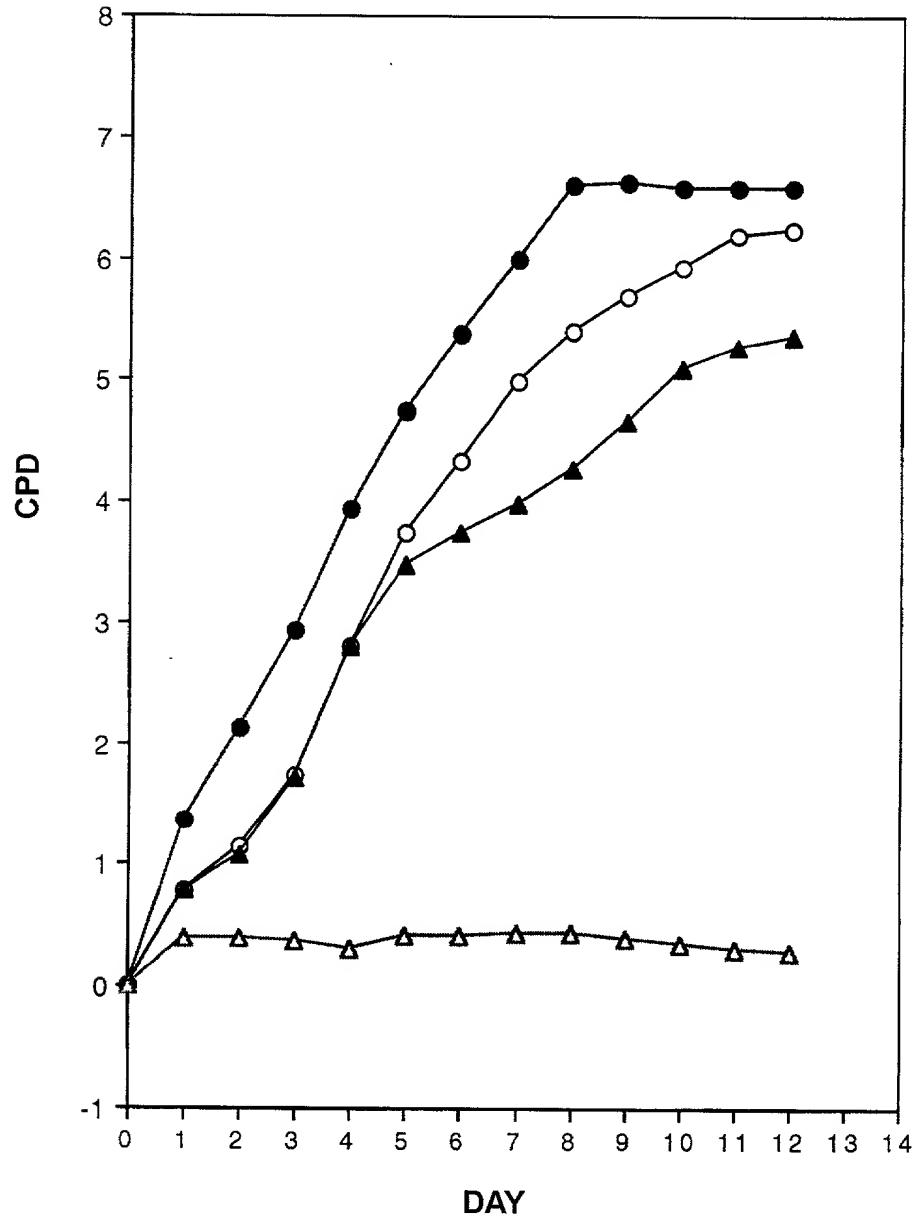
**LEGEND:**

—○— "STANDARD" MEDIUM

—●— "LOW-FE" MEDIUM

**FIGURE 32**

**LNCaP CELL GROWTH IN CAPM  $\pm$  DHT  
AND 10% FETAL BOVINE SERUM**

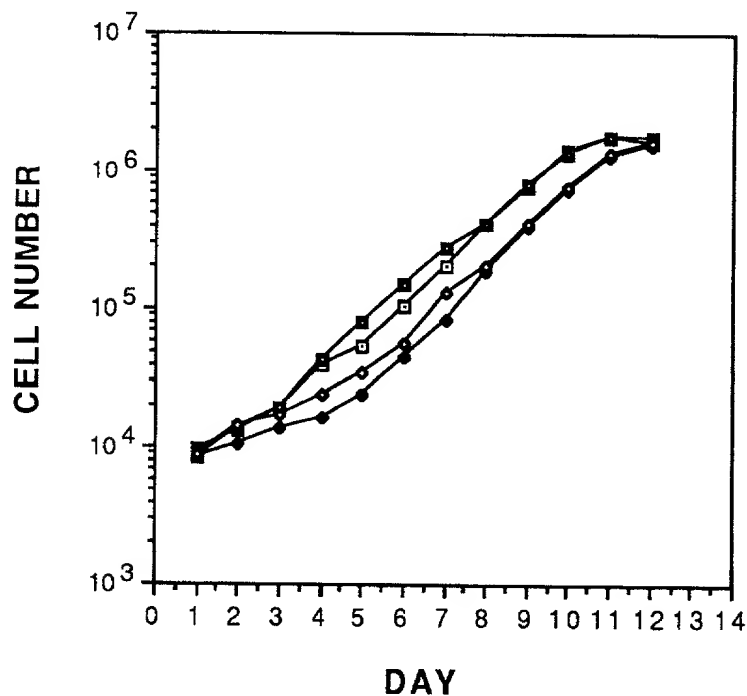


**LEGEND:**

Closed circles = Fetal bovine serum  
Open circles = CAPM + DHT  
Closed triangles = CAPM - DHT  
Open triangles = D-MEM/F12 only

**FIGURE 33**

**PC3 AND DU145 GROWTH IN SERUM - FREE  
MEDIUM VS MEDIUM WITH 10% FETAL CALF SERUM**

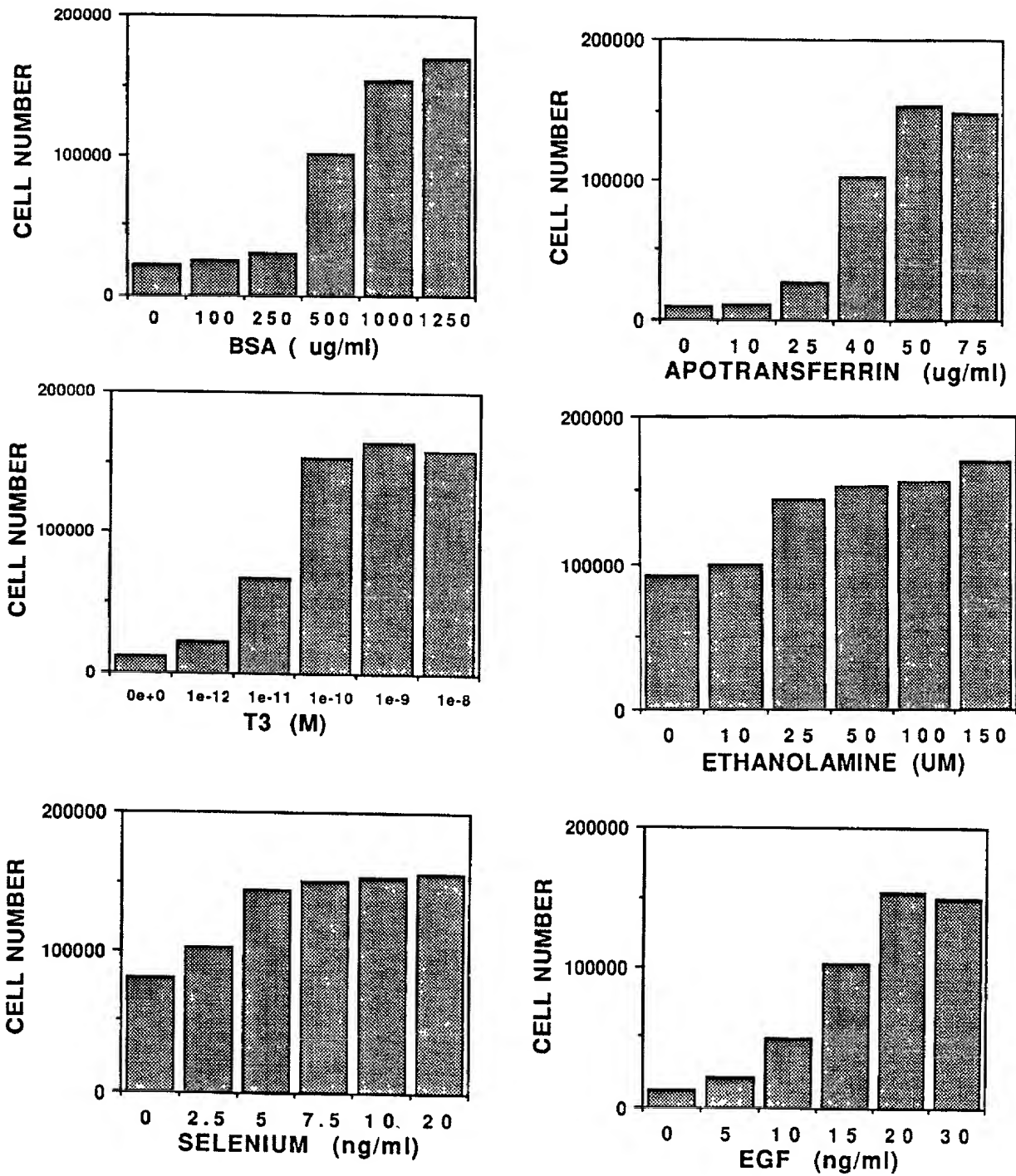


**LEGEND:**

- PC3 IN SERUM-FREE MEDIUM
- DU145 IN SERUM-FREE MEDIUM
- PC3 IN 10% FETAL CALF SERUM
- DU145 IN 10% FETAL CALF SERUM

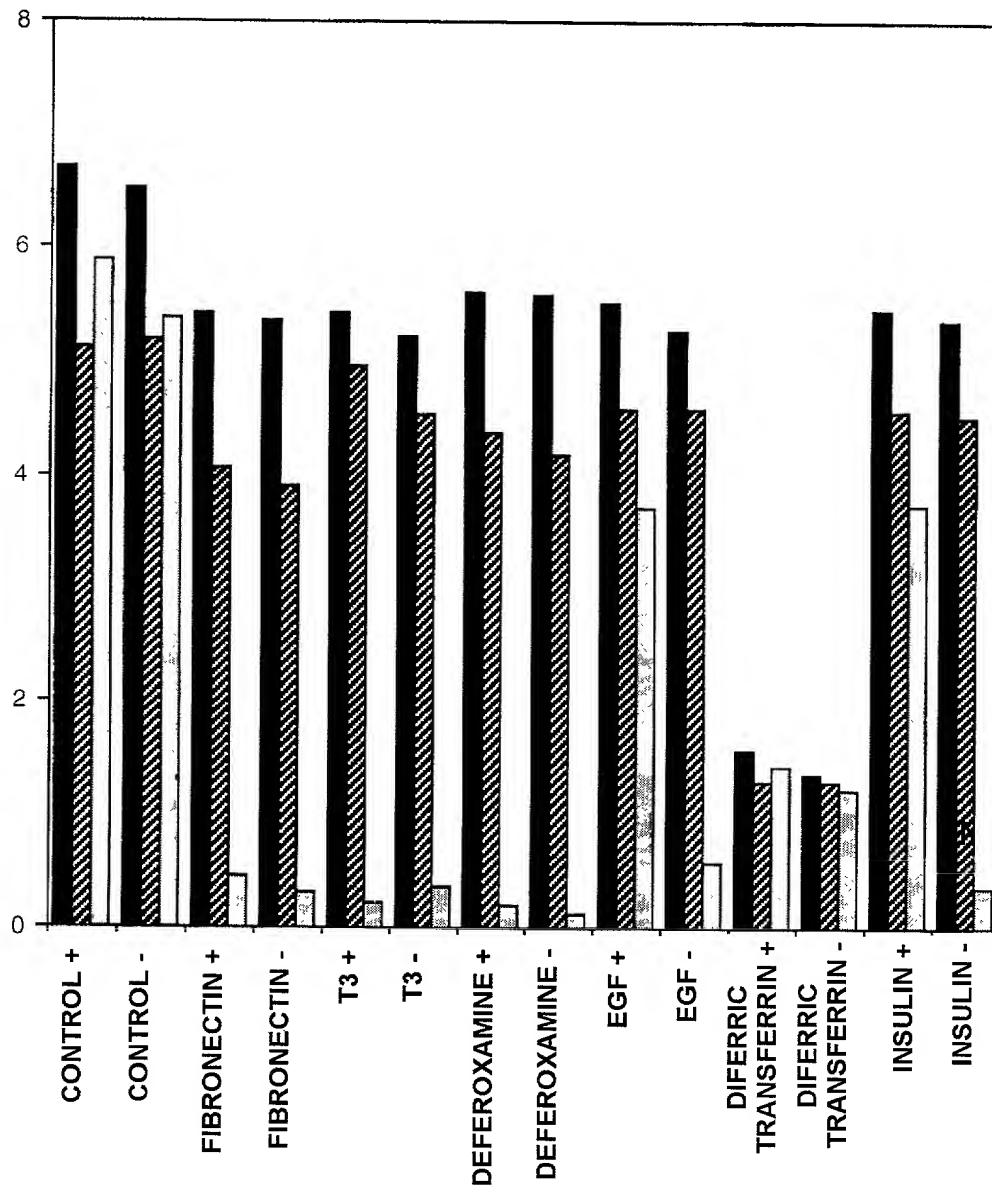
FIGURE 34

DOSE-RESPONSE EFFECTS OF INDIVIDUAL COMPONENTS  
OF CAPM SERUM-FREE MEDIUM ON LNCAP CELL GROWTH



**FIGURE 35**

**DELETIONS OF INDIVIDUAL COMPONENTS  
 OF CAPM WITH PROSTATE CANCER CELL LINES**

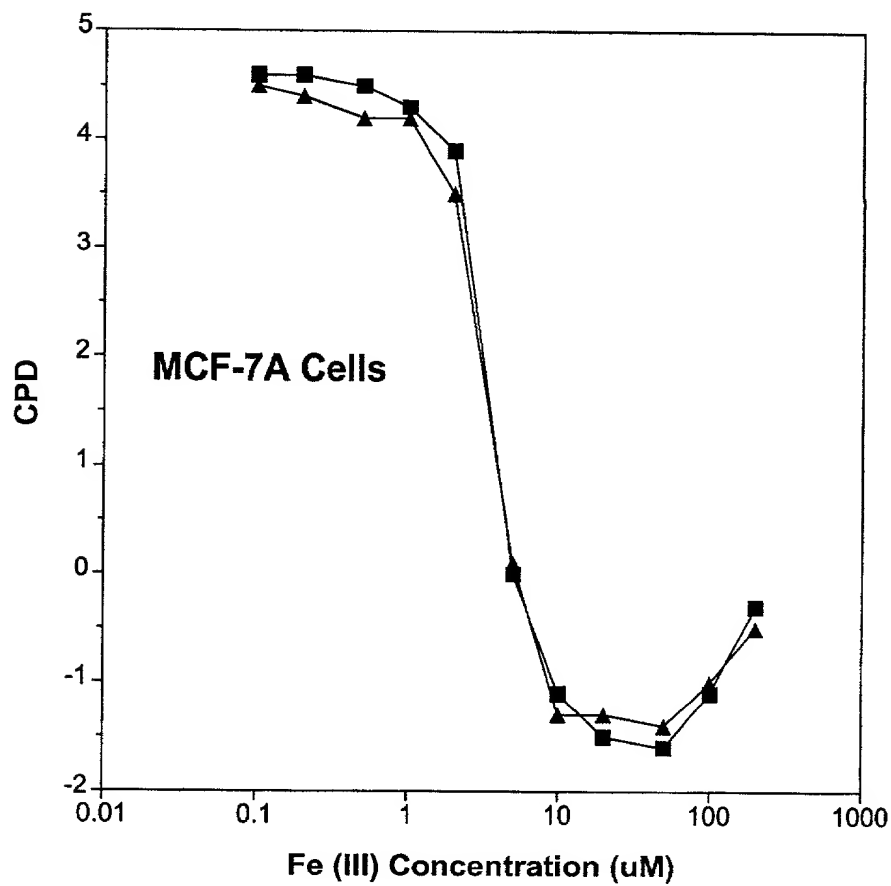


**LEGEND:**

- = PC3
- ▨ = DU145
- ▤ = LNCaP
- + = 10 nM DHT
- = NO DHT

**FIGURE 36**

**EFFECT OF FE (III) IN MCF-7A CELL GROWTH  
IN DDM-2MF DEFINED MEDIUM**

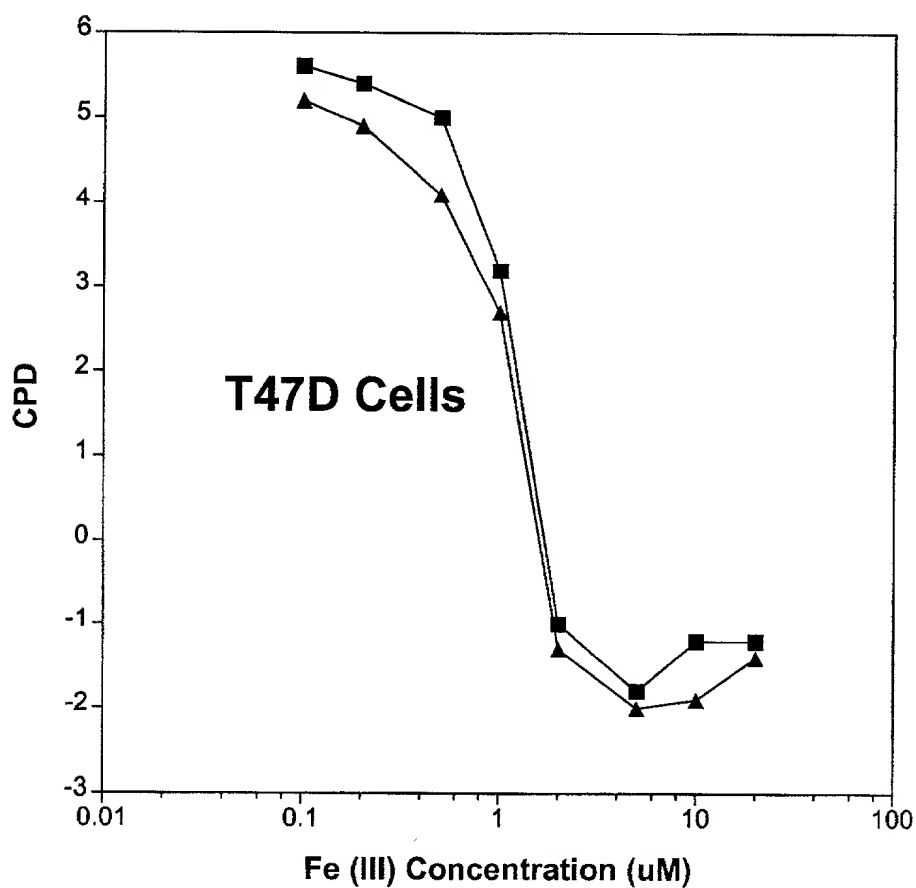


**LEGEND:**

- plus E<sub>2</sub>
- ▲— minus E<sub>2</sub>

**FIGURE 37**

**EFFECT OF FE (III) IN T47D CELL GROWTH  
IN DDM-2MF DEFINED MEDIUM**

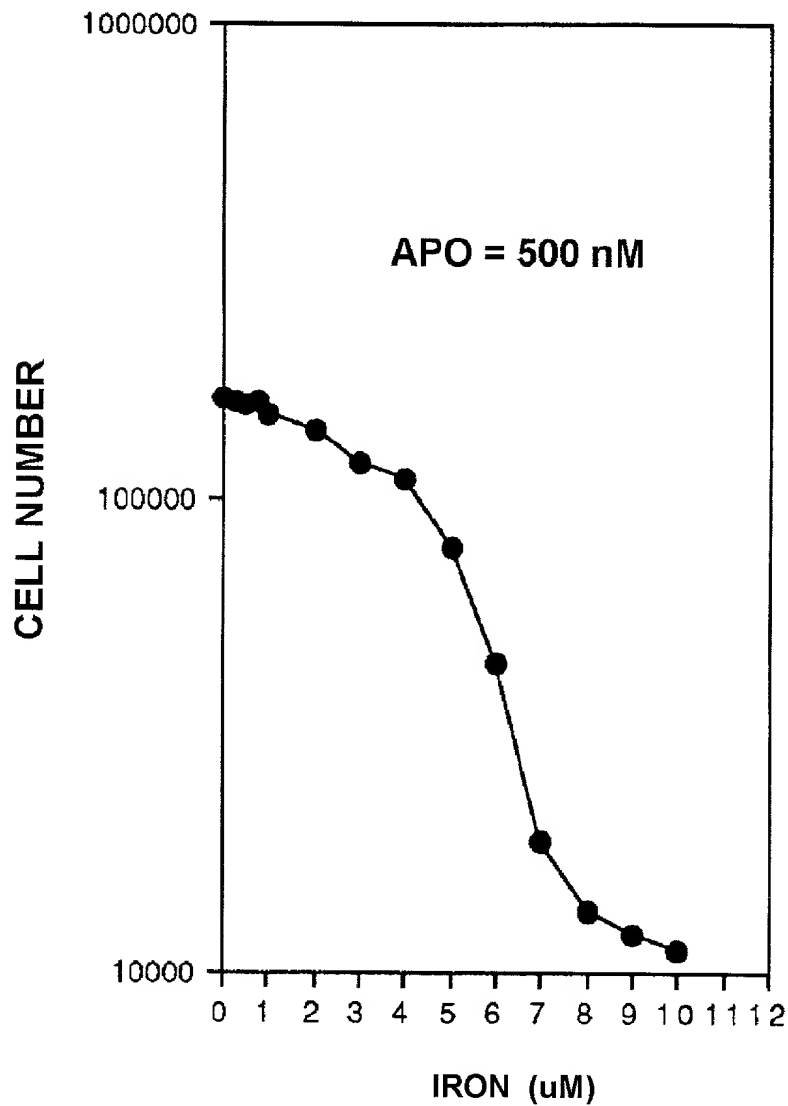


**LEGEND:**

- plus E<sub>2</sub>
- ▲— minus E<sub>2</sub>

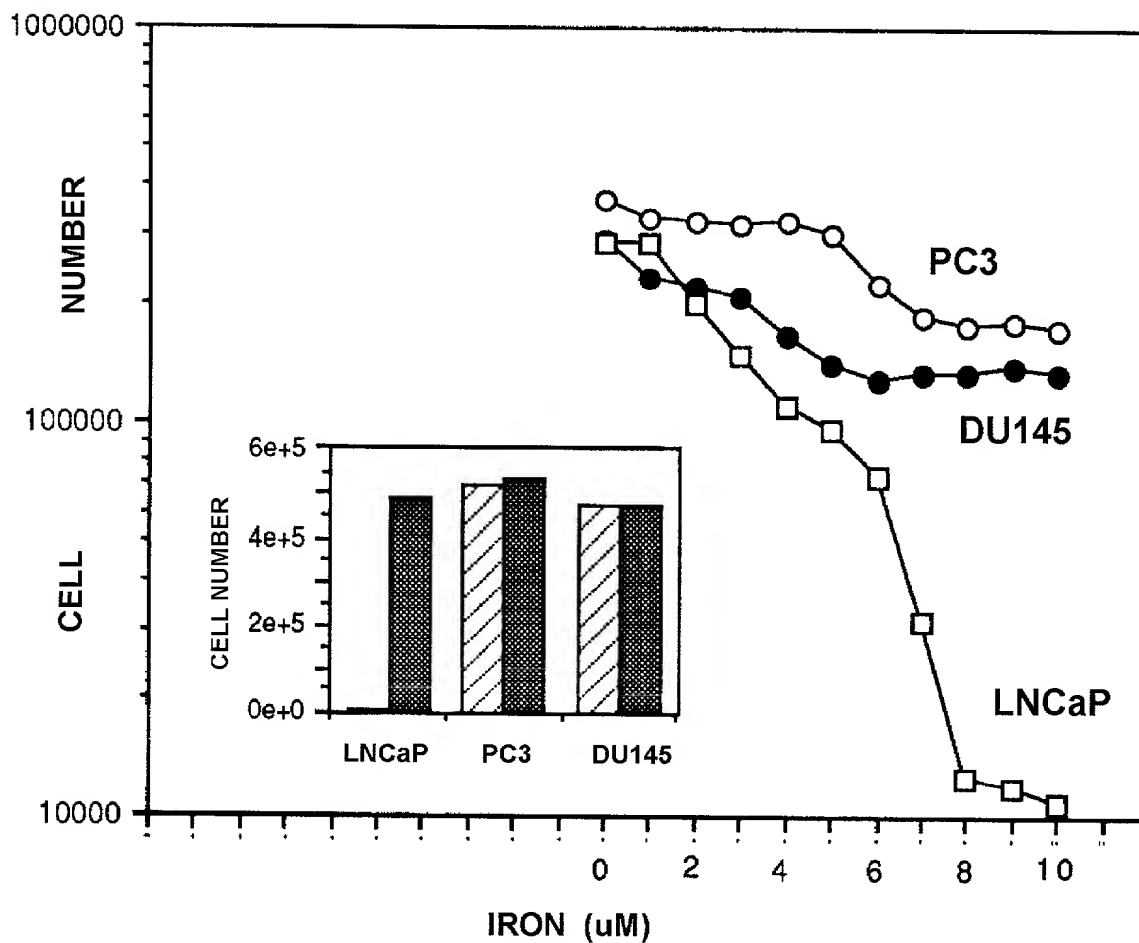
**FIGURE 38**

**EFFECTS OF INCREASING CONCENTRATIONS OF  
IRON ON LNCaP CELLS GROWN IN SERUM-FREE  
MEDIUM WITH APOTRANSFERRIN**



**FIGURE 39**

**EFFECTS OF IRON AND  $T_3$  ON THREE PROSTATIC CELL LINES IN SERUM-FREE MEDIUM**



**INSERT:**

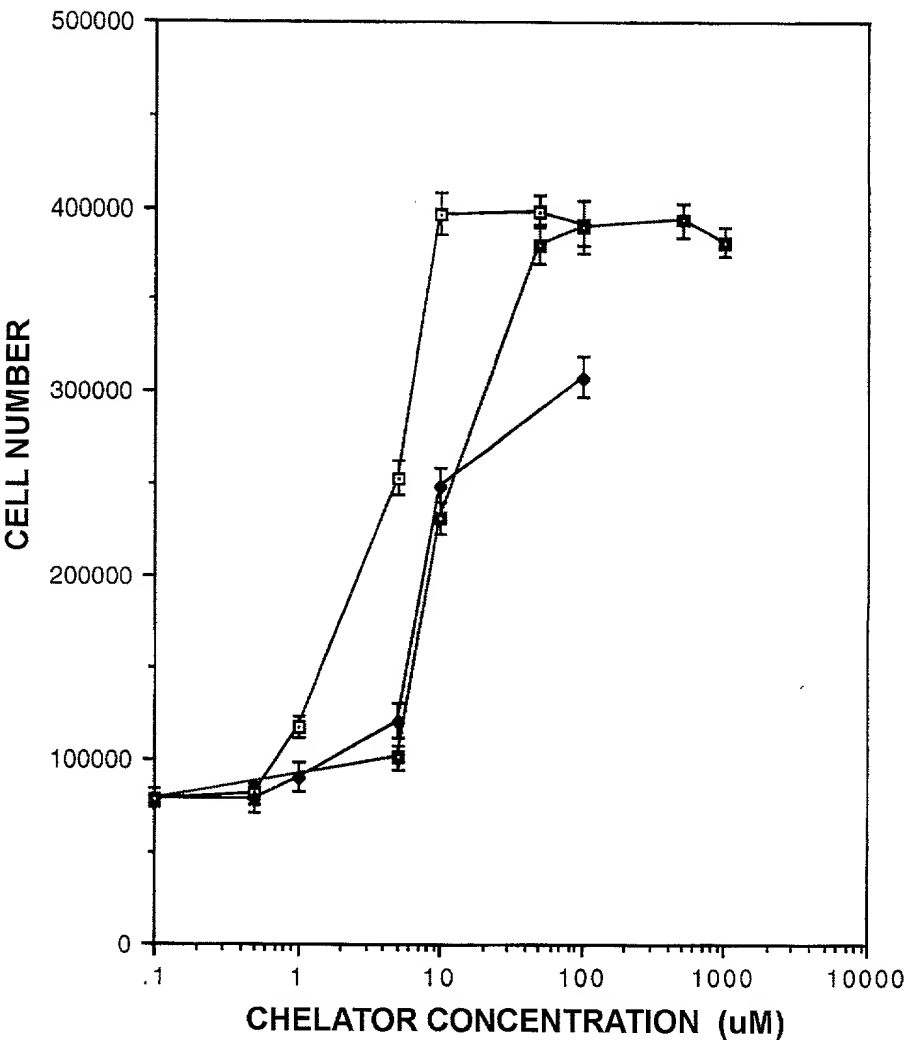
**DARK BARS = GROWTH IN CAPM PLUS  $T_3$**

**LIGHT (HATCHED) BARS = GROWTH IN CAPM MINUS  $T_3$**

**NOTE THE STRIKING DEPENDENCE OF LNCaP CELLS ON  $T_3$**

FIGURE 40

EFFECT OF CHELATORS ON SERUM-FREE T47D  
GROWTH UNDER HIGH IRON CONDITIONS

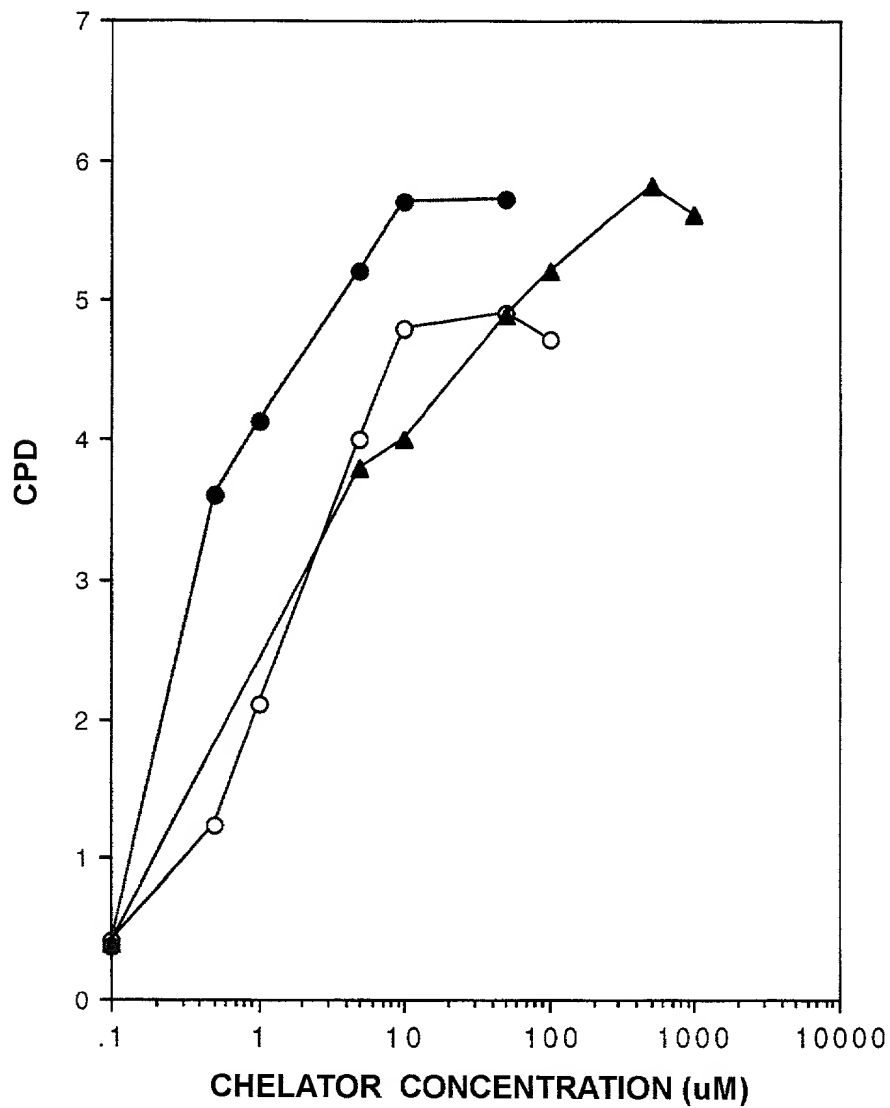


LEGEND:

- DEFEROXAMINE
- ♦— EDTA
- CITRATE

**FIGURE 41**

**EFFECT OF CHELATORS ON SERUM-FREE LNCaP  
GROWTH UNDER HIGH IRON CONDITIONS**



**LEGEND:**

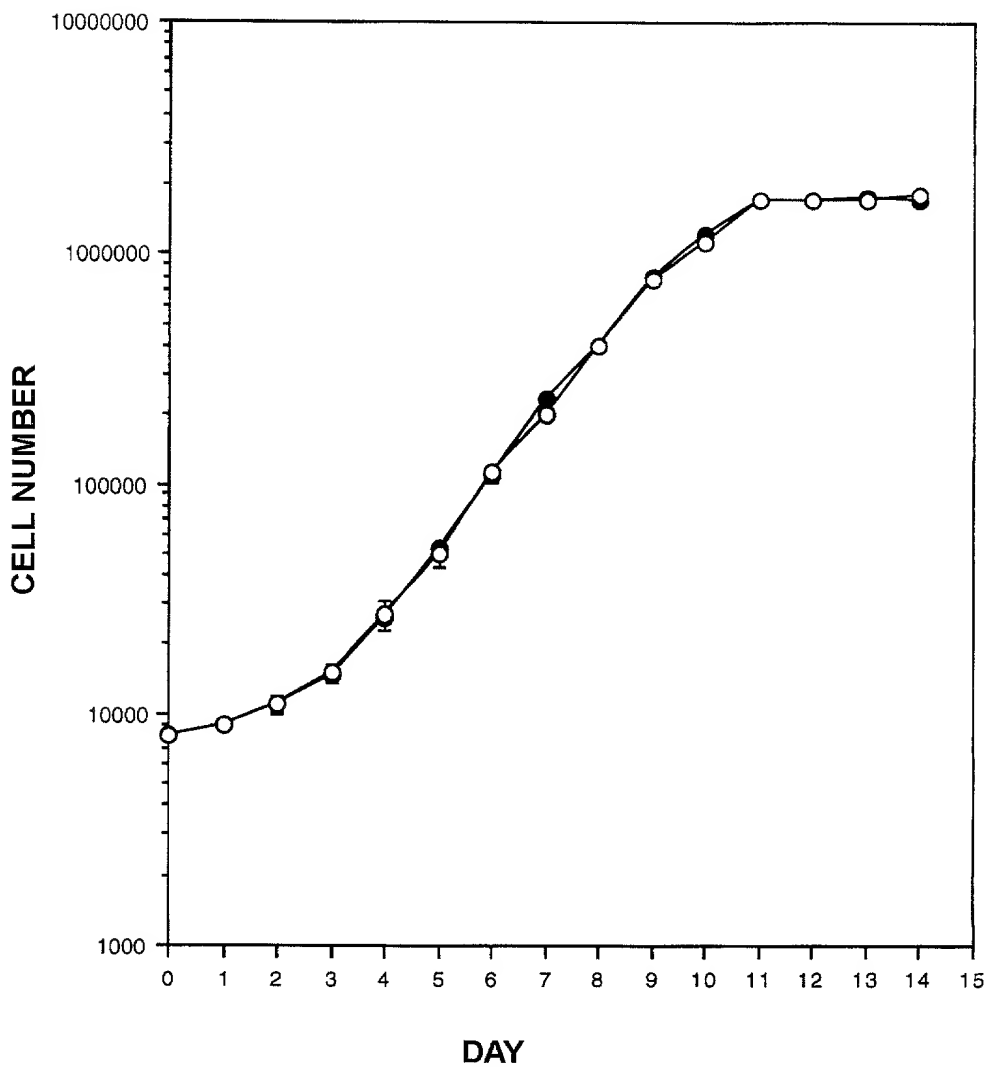
Closed circles = Deferoxamine

Open circles = Citrate

Closed triangles = EDTA

**FIGURE 42**

**DU145 GROWTH IN SERUM-FREE MEDIUM  
BASED ON "LOW FE" OR "STANDARD" MEDIUM**



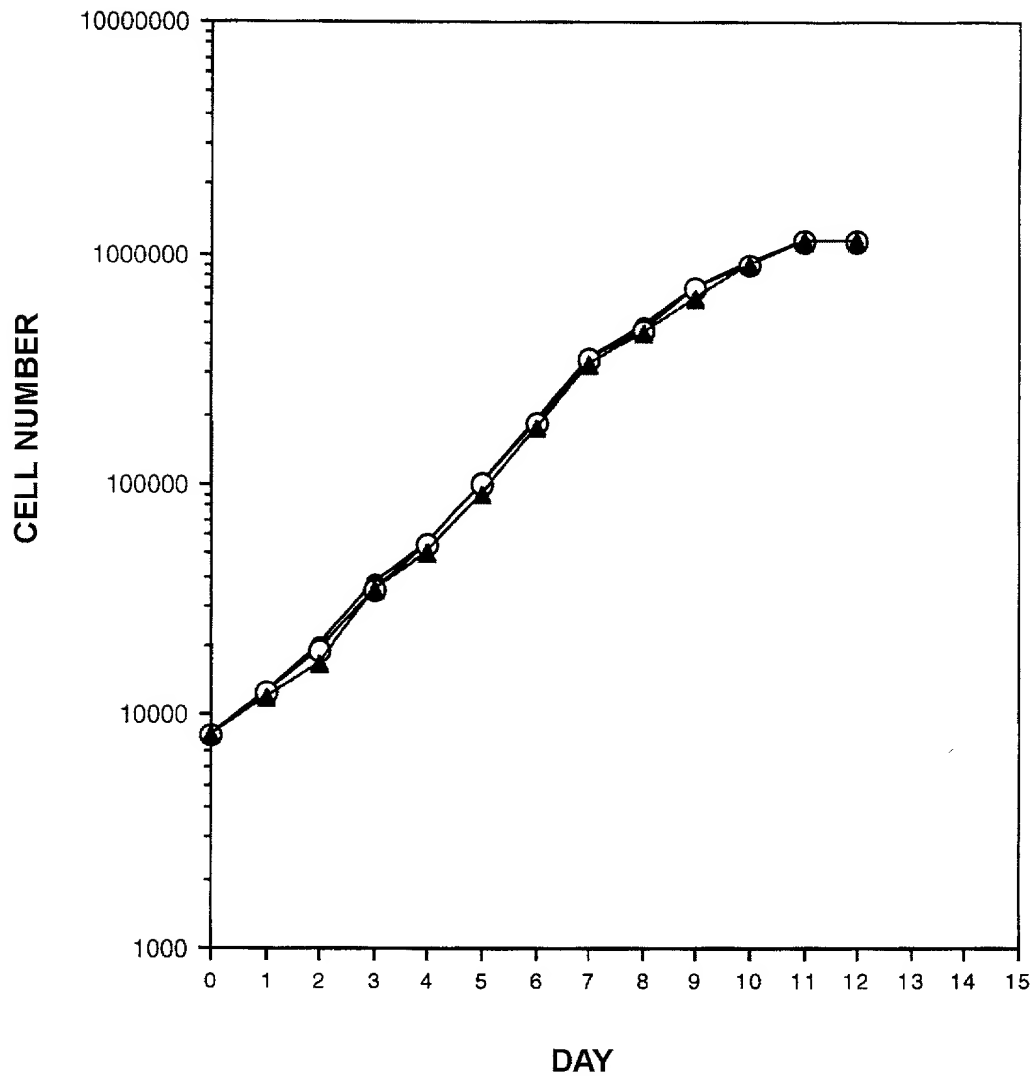
**LEGEND:**

Open circles = "Low Fe" medium

Closed circles = "Standard" medium

**FIGURE 43**

**PC3 GROWTH IN SERUM-FREE MEDIUM  
BASED ON "LOW FE" OR "STANDARD" MEDIUM**



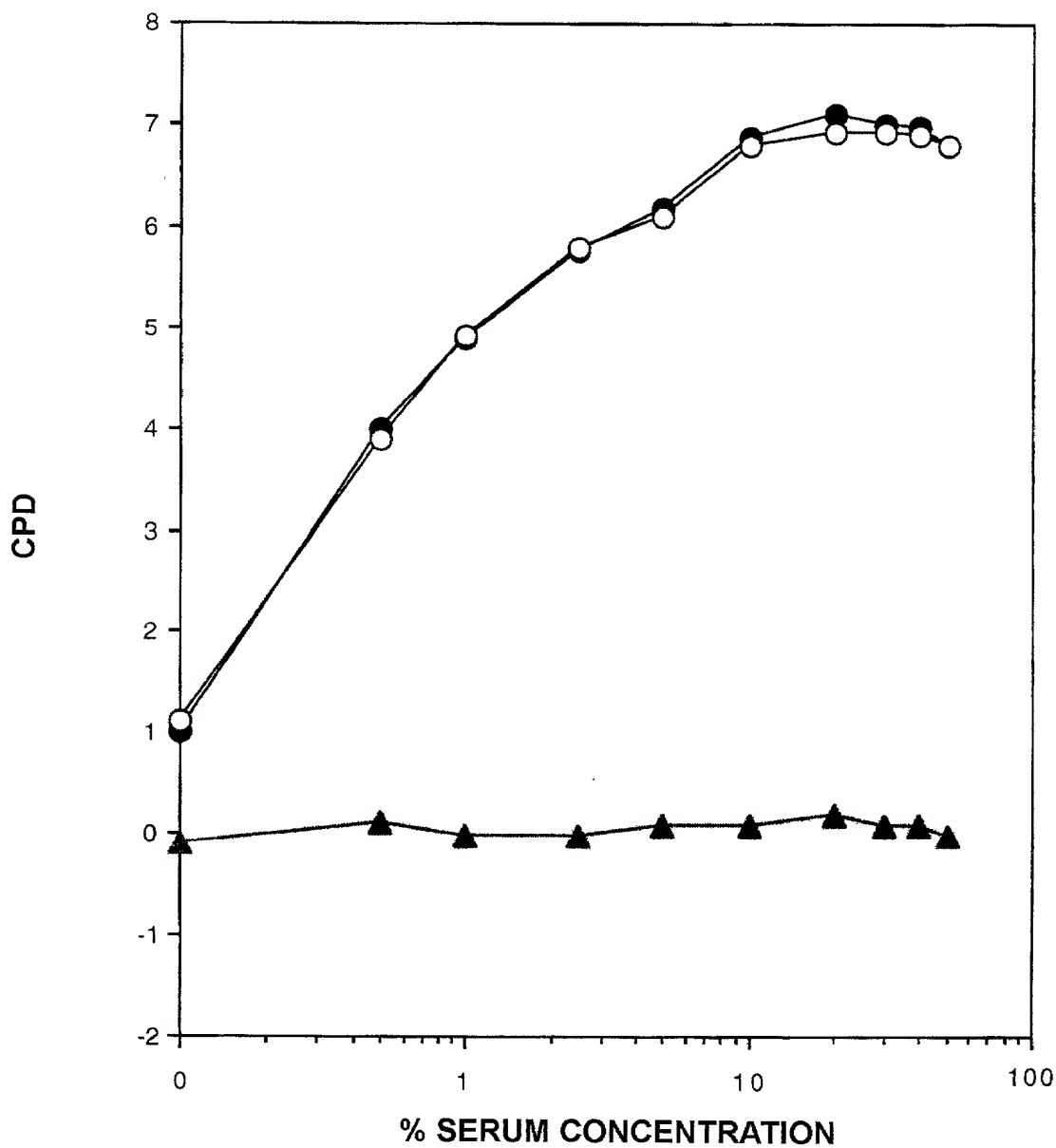
**LEGEND:**

Open circles = "Low Fe" medium

Closed triangles = "Standard" medium

**FIGURE 44**

**CDE HORSE SERUM TITRATION ON DU145 CELLS**

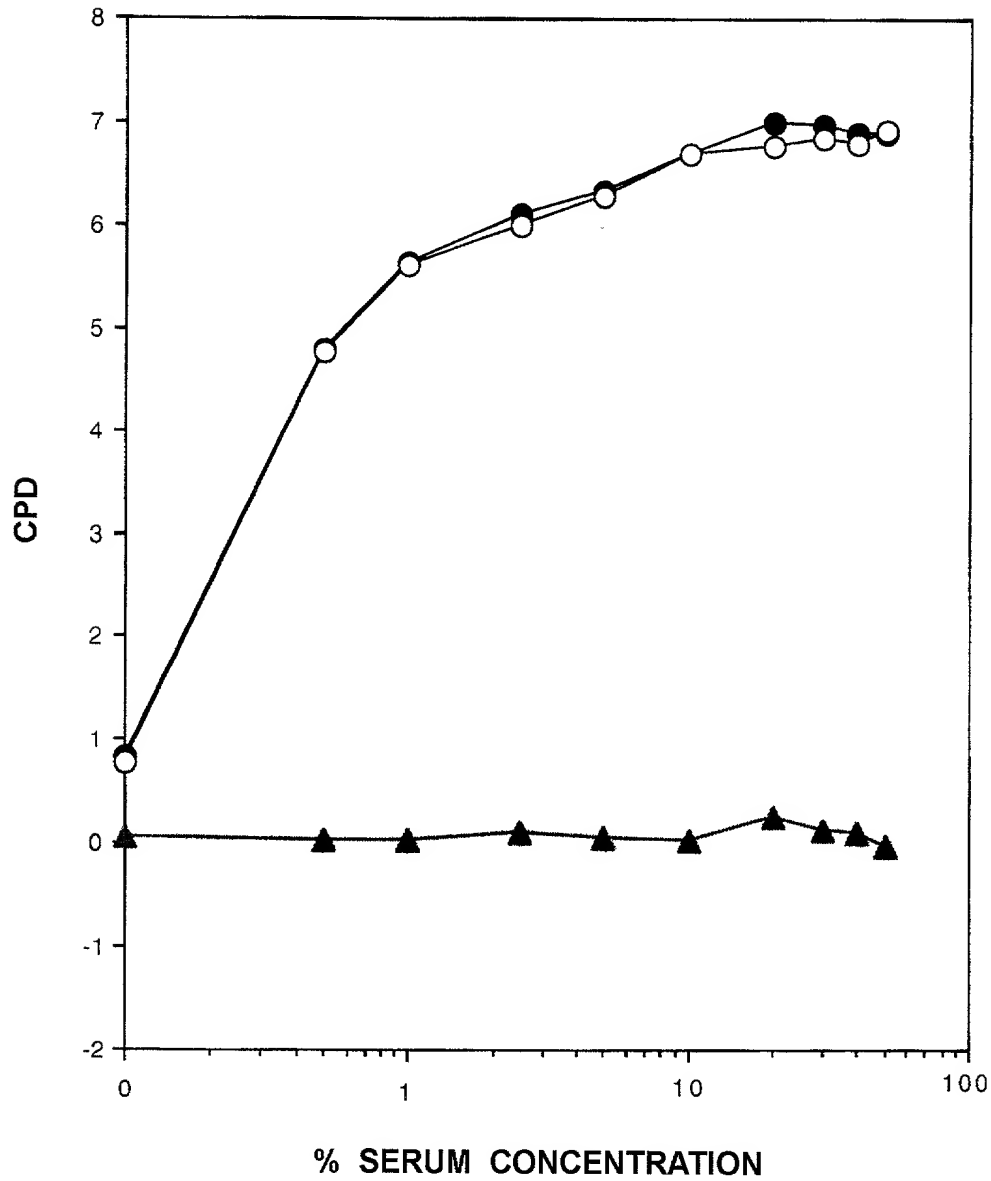


**LEGEND:**

- = + 10 nM DHT
- = STEROID FREE
- ▲— = ANDROGENIC EFFECT

**FIGURE 45**

**CDE HORSE SERUM TITRATION ON PC3 CELLS**

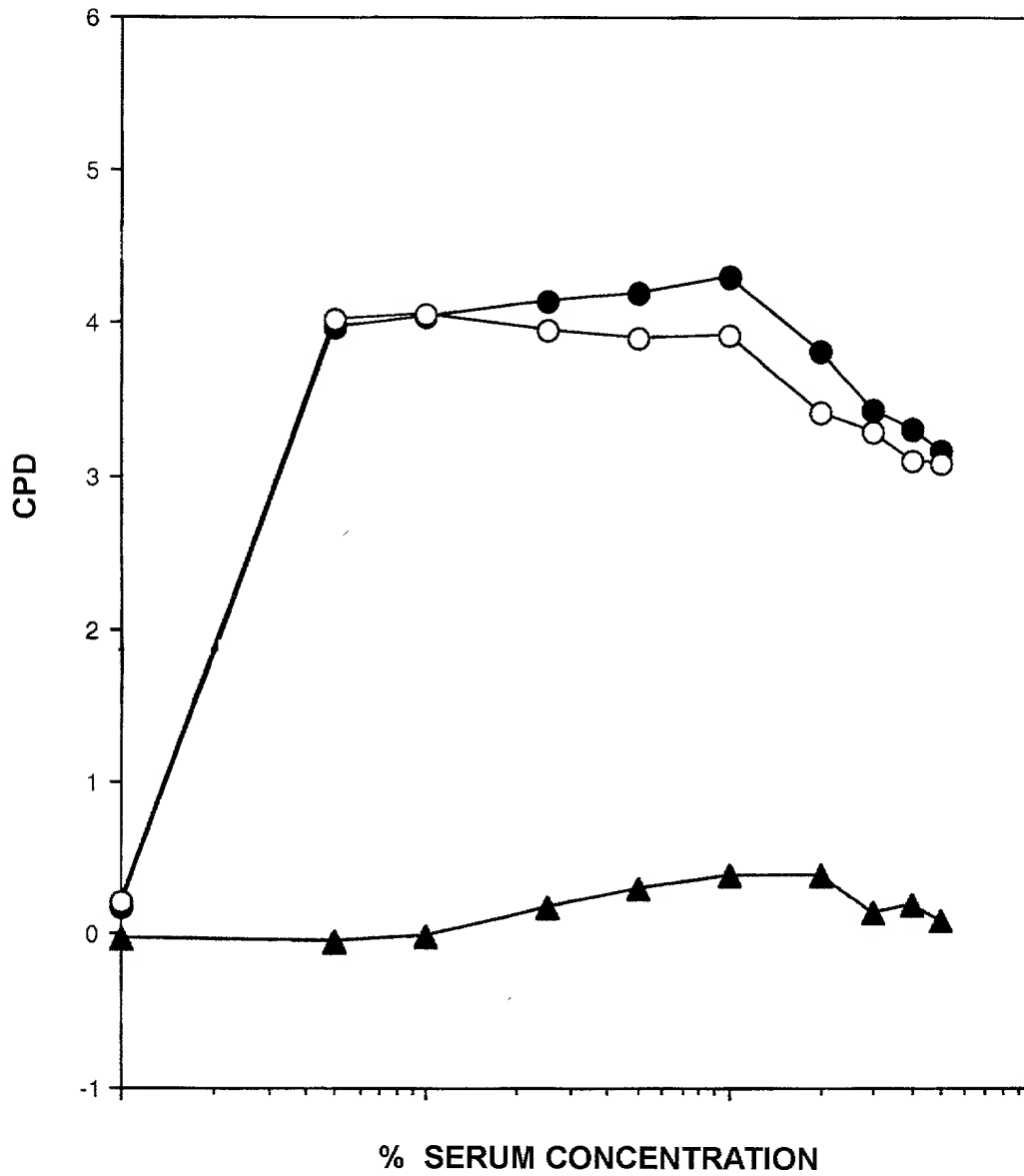


**LEGEND:**

- = + 10 nM DHT
- = STEROID FREE
- ▲— = ANDROGENIC EFFECT

**FIGURE 46**

**CDE HORSE SERUM TITRATION ON ALVA-41 CELLS**

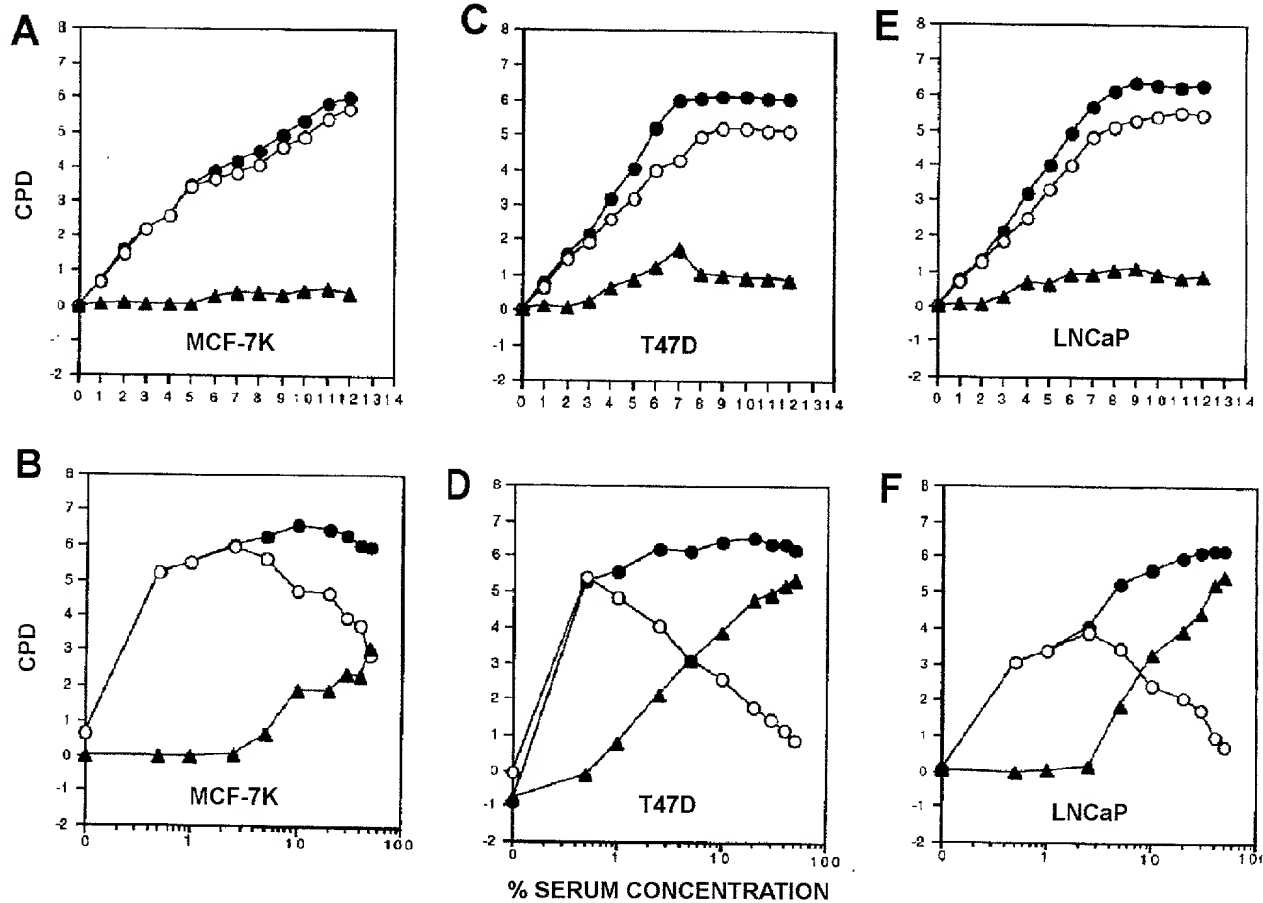


**LEGEND:**

- = + 10 nM DHT
- = STERIOD FREE
- ▲ = ANDROGENIC EFFECT

**FIGURE 47**

**EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE HUMAN TUMOR CELL GROWTH**

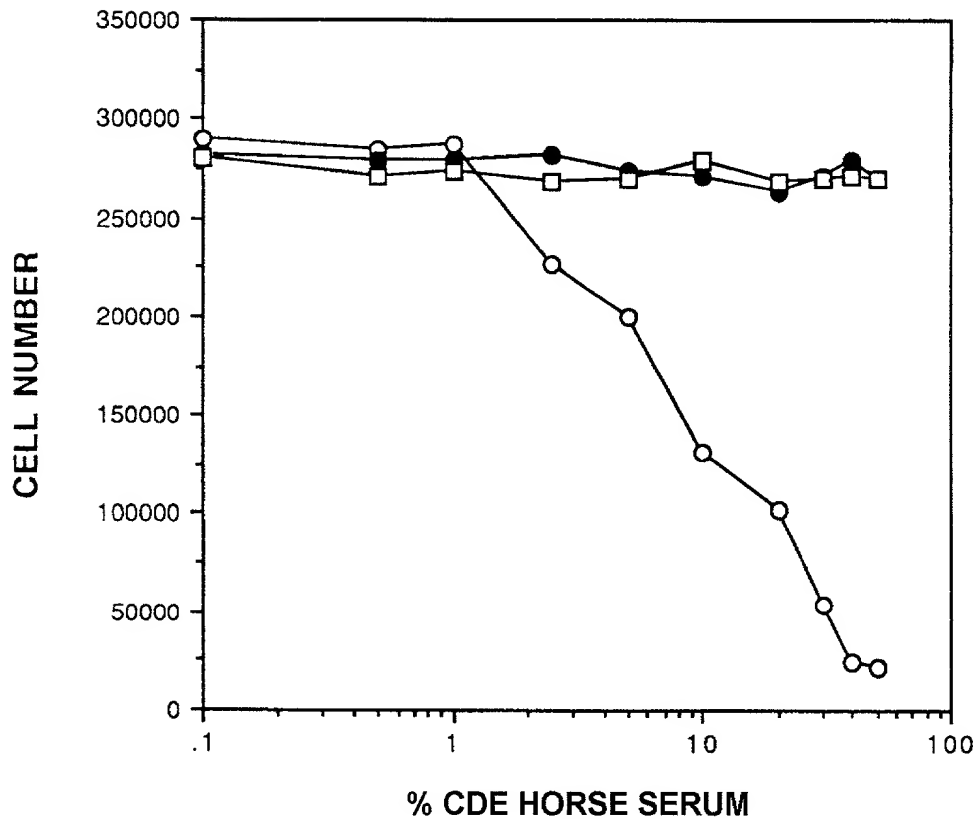


The cells were grown in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

- (A) MCF-7K cell growth was measured daily in serum-free defined DDM-2MF with 10 nM E<sub>2</sub> (closed circles) and without steroid (open circles) E<sub>2</sub>. Triangles = estrogenic effect.  
 (B) MCF-7K cell growth measured after 12 d in D-MEM-F-12 supplemented with the designated concentrations of serum with E<sub>2</sub> (closed circles) and without steroid (open circles). The estrogenic effect is shown by triangles.  
 (C) and (D) show the same experiments as in (A) and (B), respectively, except with T47D cells.  
 (E) and (F) show the same experiments as in (A) and (B), respectively, except with LNCaP cells. In (E) the serum-free medium was CAPM.

**FIGURE 48**

**CDE HORSE SERUM TITRATION ON LNCaP  
GROWTH IN SERUM FREE CONDITIONS**

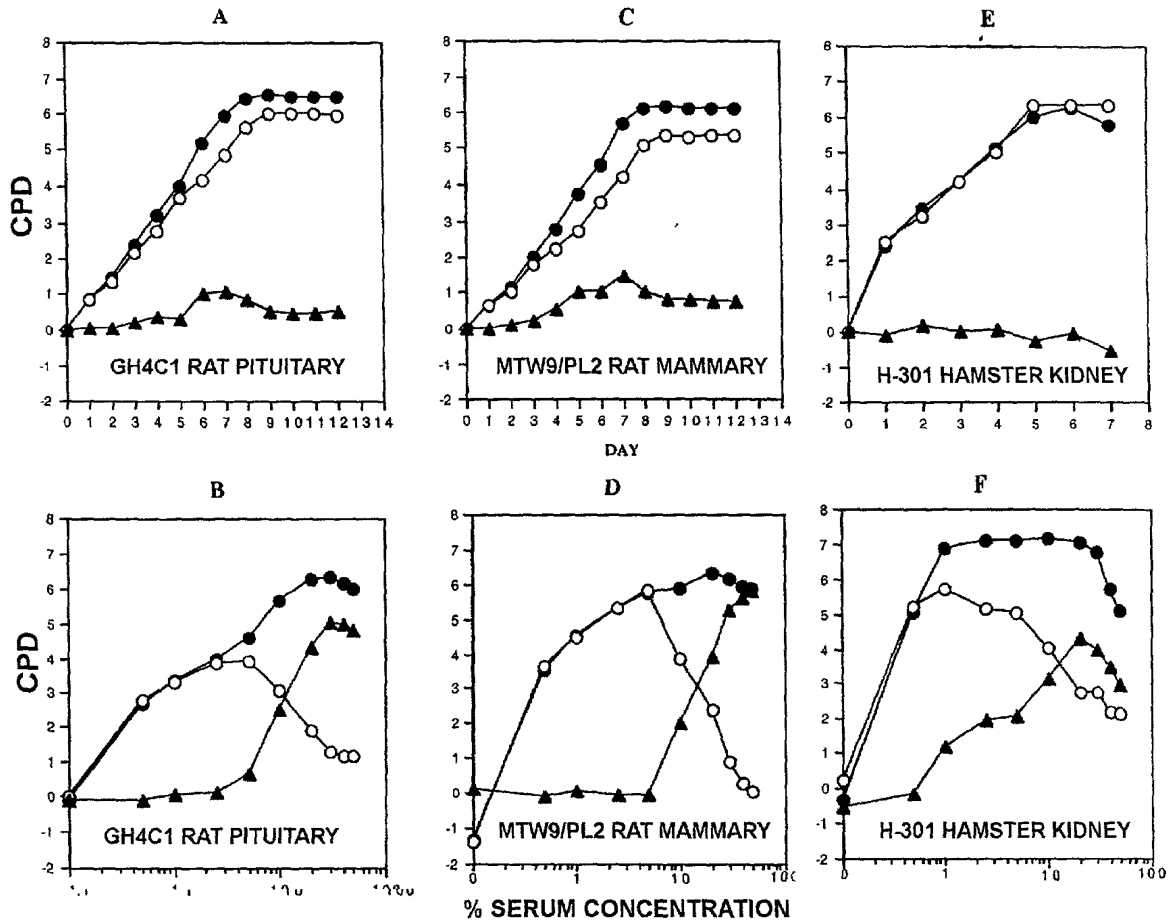


**LEGEND:**

- NO STEROID
- + E<sub>2</sub>
- + DHT

**FIGURE 49**

**EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE RODENT TUMOR CELL GROWTH**

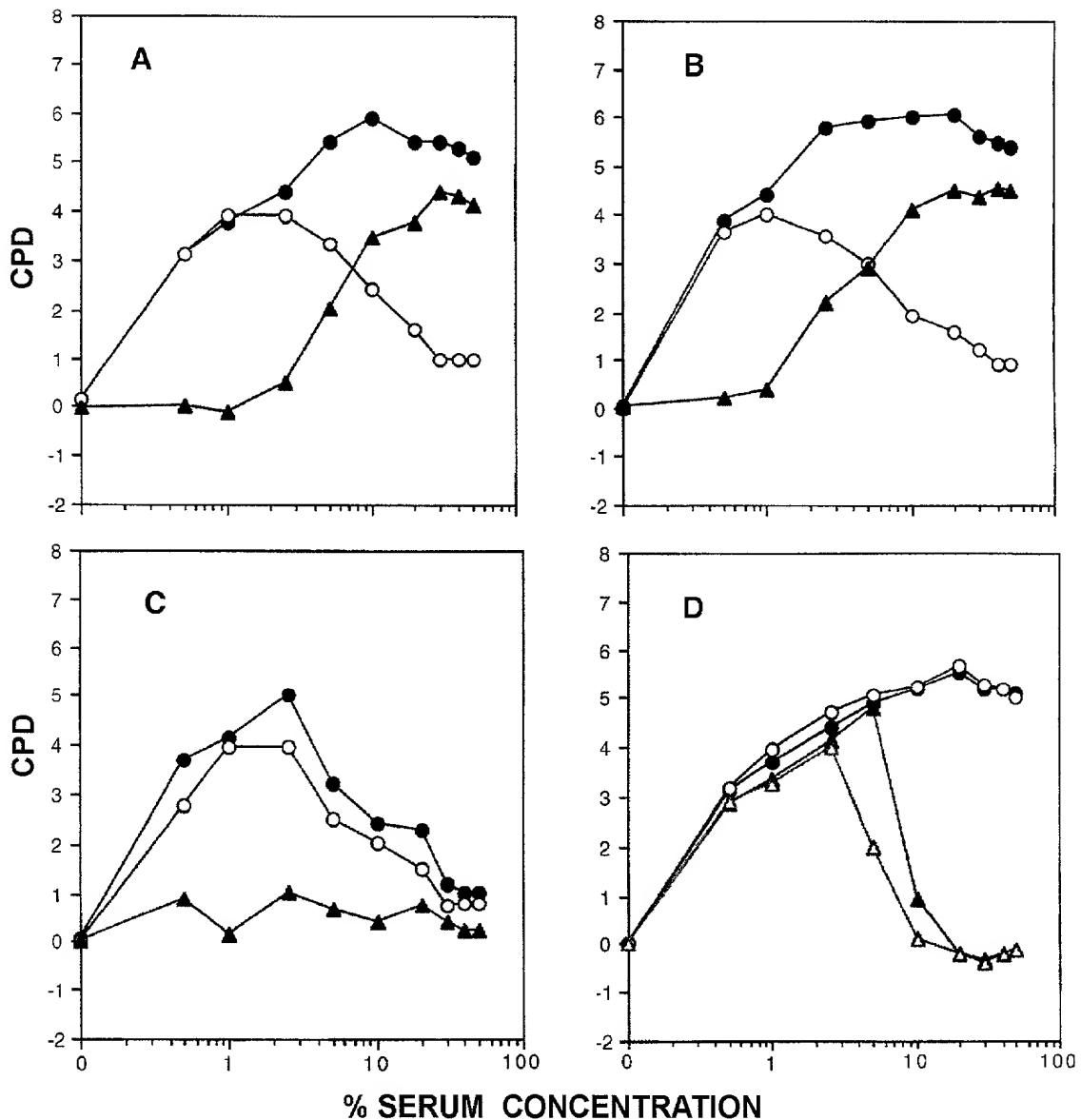


Comparison of the effects of estrogen on steroid hormone-responsive rodent tumor cell growth in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) GH<sub>4</sub>C<sub>1</sub> rat pituitary tumor cell growth measured daily in serum-free PCM-9 with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (open circles). The estrogenic effect is shown by triangles.  
 (B) GH<sub>4</sub> C<sub>1</sub> cell growth measured after 9 d in D-MEM-F-12 supplemented with the designated concentrations of CDE horse serum with E<sub>2</sub> (closed circles) and without E<sub>2</sub> (open circles). The estrogenic effect is shown by triangles.  
 (C) and (D) show the same experiments as in (A) and (B) respectively, but with the MTW9/PL2 rat mammary tumor cells. The serum-free medium in (D) was DDM-2A.  
 (E) and (F) show the same experiments as in (A) and (B), respectively, except with the H-301 hamster kidney tumor cells. In (E) the serum-free medium was CAPM.

**FIGURE 50**

**THE EFFECT OF DHT, E<sub>2</sub>, AND DES ON  
 LNCaP CELLS GROWN IN CDE HORSE SERUM**

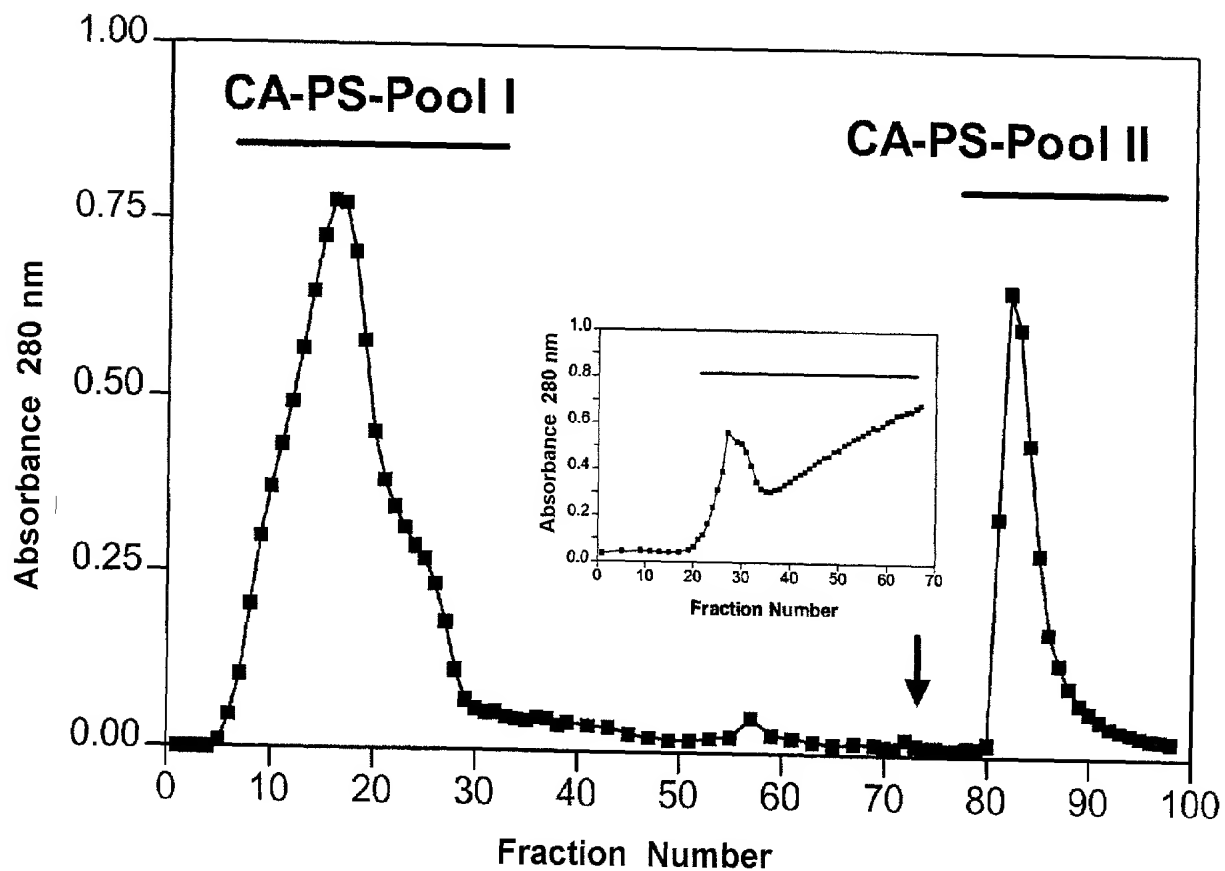


**LEGEND:**

- (A) Open circles = - DHT  
 Closed circles = + DHT  
 Closed triangles = Androgenic effect
- (B) Open circles = - E<sub>2</sub>  
 Closed circles = + E<sub>2</sub>  
 Closed triangles = Estrogenic effect
- (C) Open circles = - DES  
 Closed circles = + DES  
 Closed triangles = Estrogenic effect
- (D) Open circles = DHT & DES  
 Closed circles = E<sub>2</sub> & DES  
 Open triangles = No additions  
 Closed triangles = DES only

**FIGURE 51**

**PHENYL SEPHAROSE ELUTION OF  
CBG (CA-PS-POOL 1) AND SHBG-LIKE (CA-PS-POOL 11)**



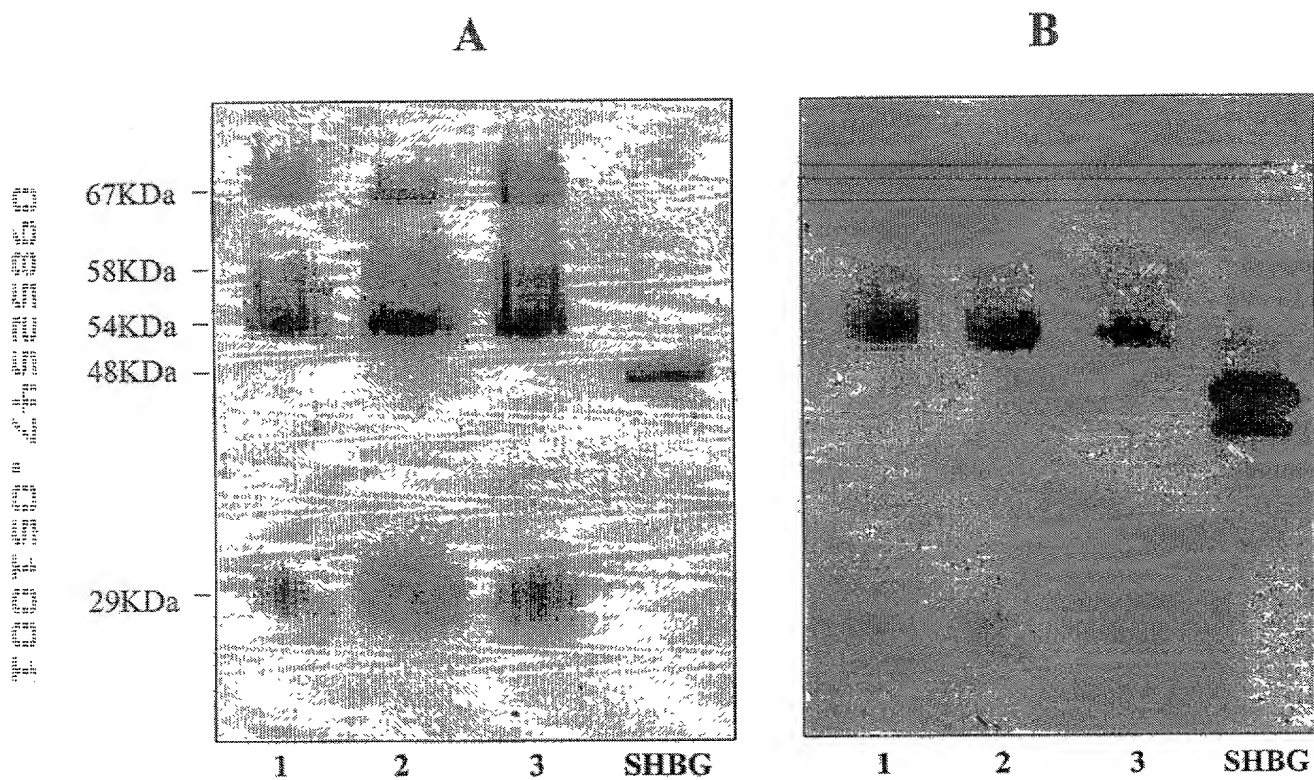
**ARROW = ELUTION WITH 40% ETHYLENE GLYCOL**

**INSERT: CORTISOL AFFINITY COLUMN ELUTION**

**BARS = POOLED ACTIVE FRACTION**

## FIGURE 52

### SDS PAGE (A) AND WESTERN ANALYSIS (B) OF THREE PREPARATIONS OF CA-PS-POOL II VS HUMAN SHBG

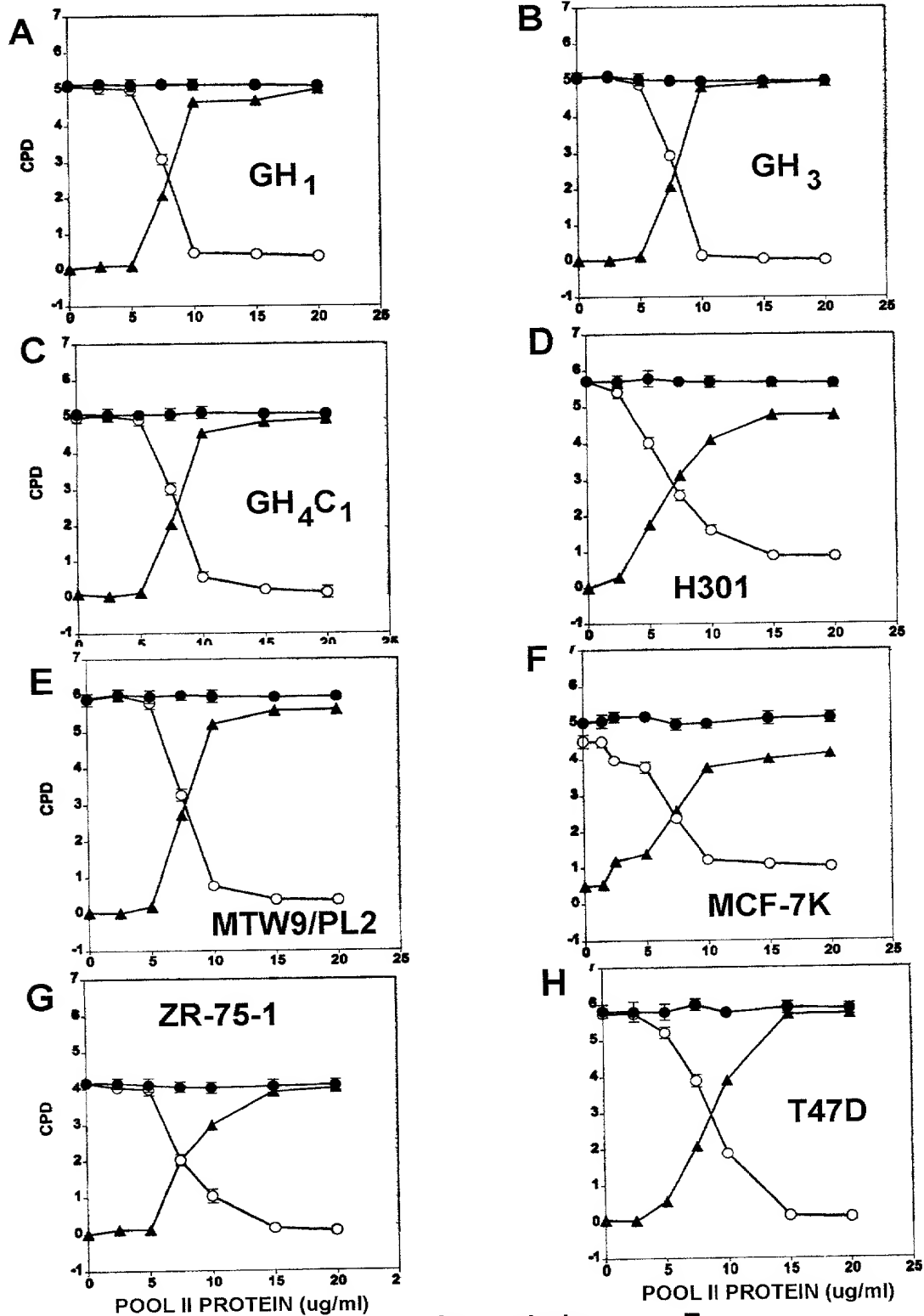


LANES 1, 2, AND 3 = 10 ug each of CA-PS-POOL II

LANE "SHBG" = 10 mg of purified protein

**FIGURE 53**

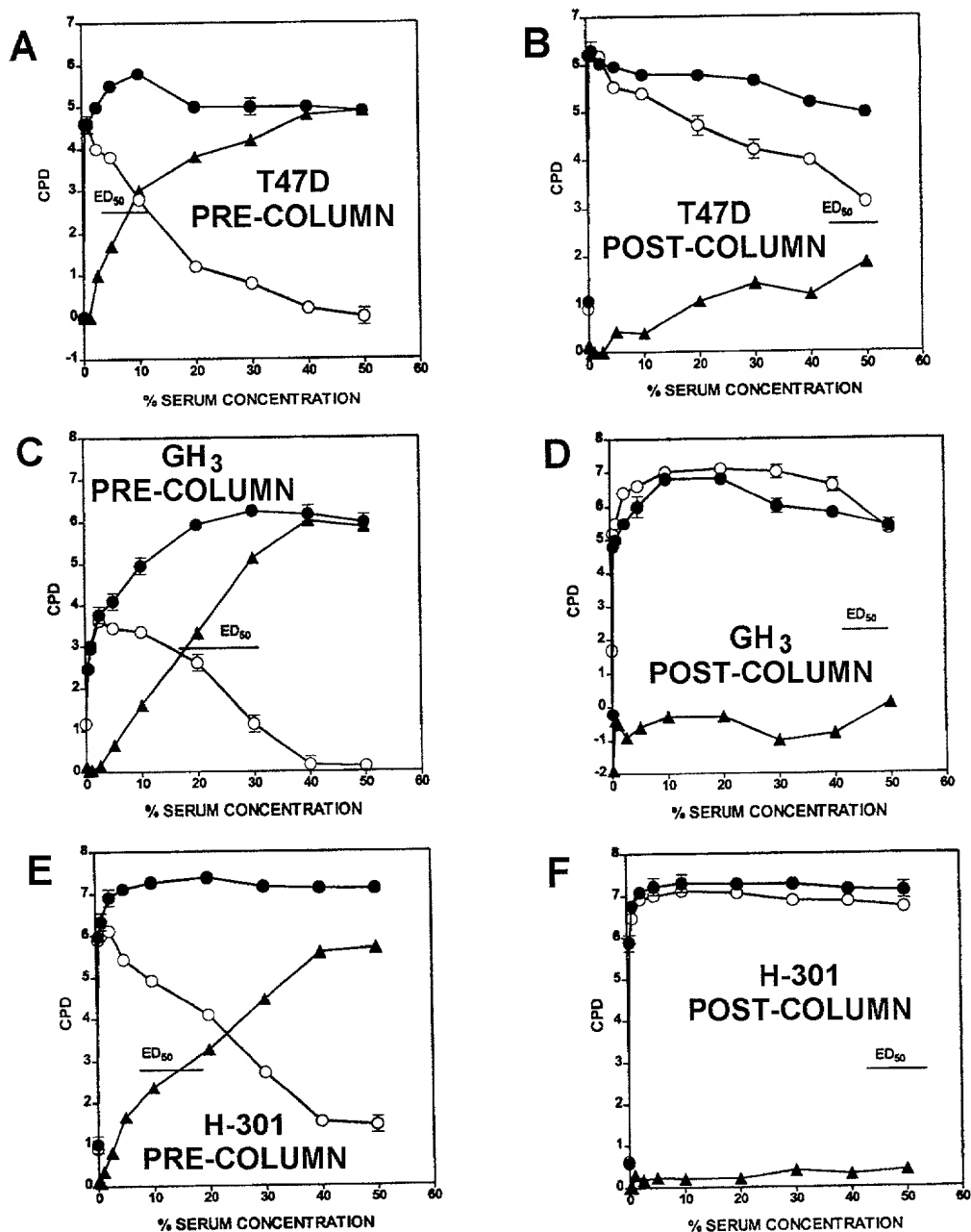
**ASSAY OF CA-PS-POOL II ESTROGEN REVERSIBLE  
 INHIBITORY ACTIVITY WITH SEVERAL ER<sup>+</sup>CELL LINES**



**LEGEND:**

Open circles = - E<sub>2</sub>  
 Closed circles = + E<sub>2</sub>  
 Closed triangles = Estrogenic effect

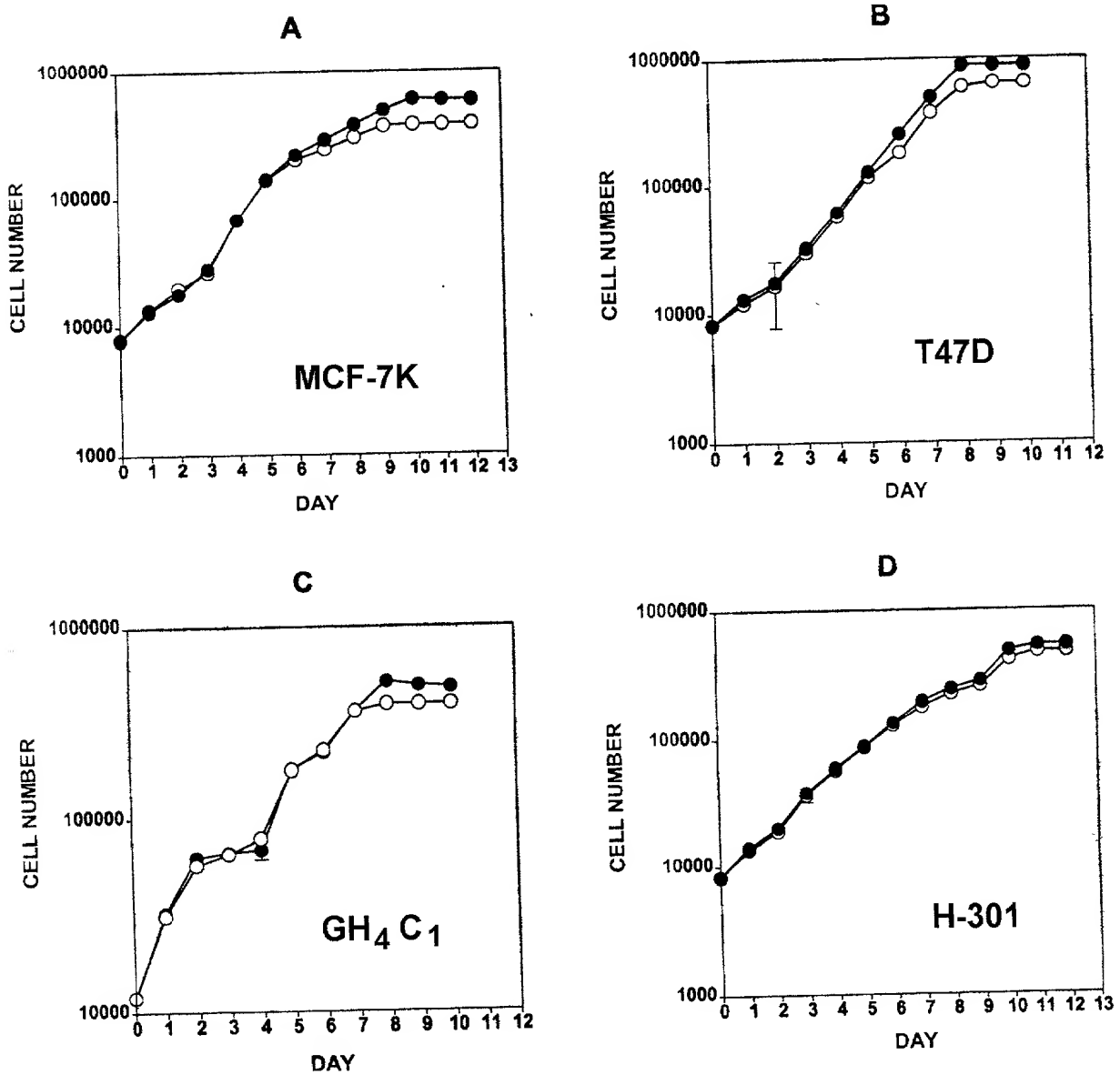
# **FIGURE 54** **CORTISOL-AGAROSE AFFINITY REMOVAL** **OF THE INHIBITOR FROM CDE-SERUM**



**LEGEND:**      Open circles = – E<sub>2</sub>  
                   Closed circles = + E<sub>2</sub>  
                   Closed triangles = Estrogenic effect

**FIGURE 55**

**GROWTH OF ER<sup>+</sup> CELL LINES IN  
 SERUM-FREE MEDIUM  $\pm$  E<sub>2</sub>**



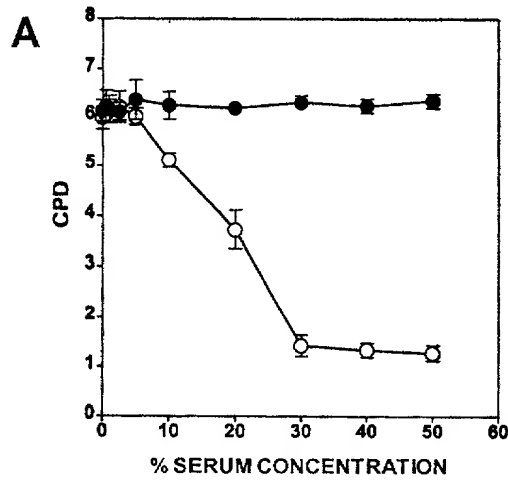
**LEGEND:**

Closed circles = + E<sub>2</sub>

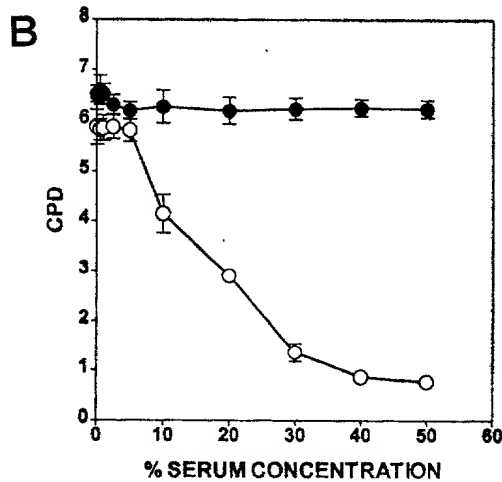
Open circles = - E<sub>2</sub>

**FIGURE 56**

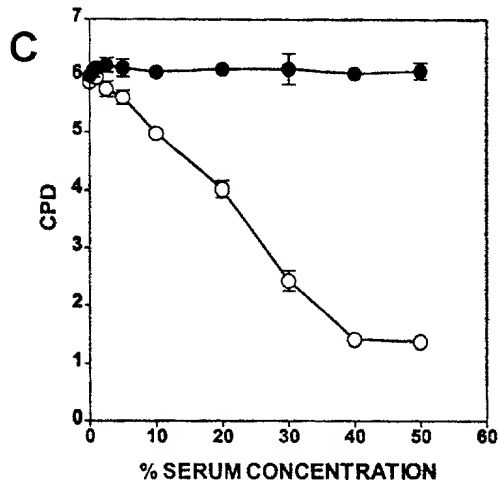
**EFFECT OF CDE-SERUM ON ESTROGEN RESPONSIVE  
 GROWTH OF THREE ER<sup>+</sup> CANCER CELL LINES IN SFM**



**A =  
 T47D IN DDM-2MF**



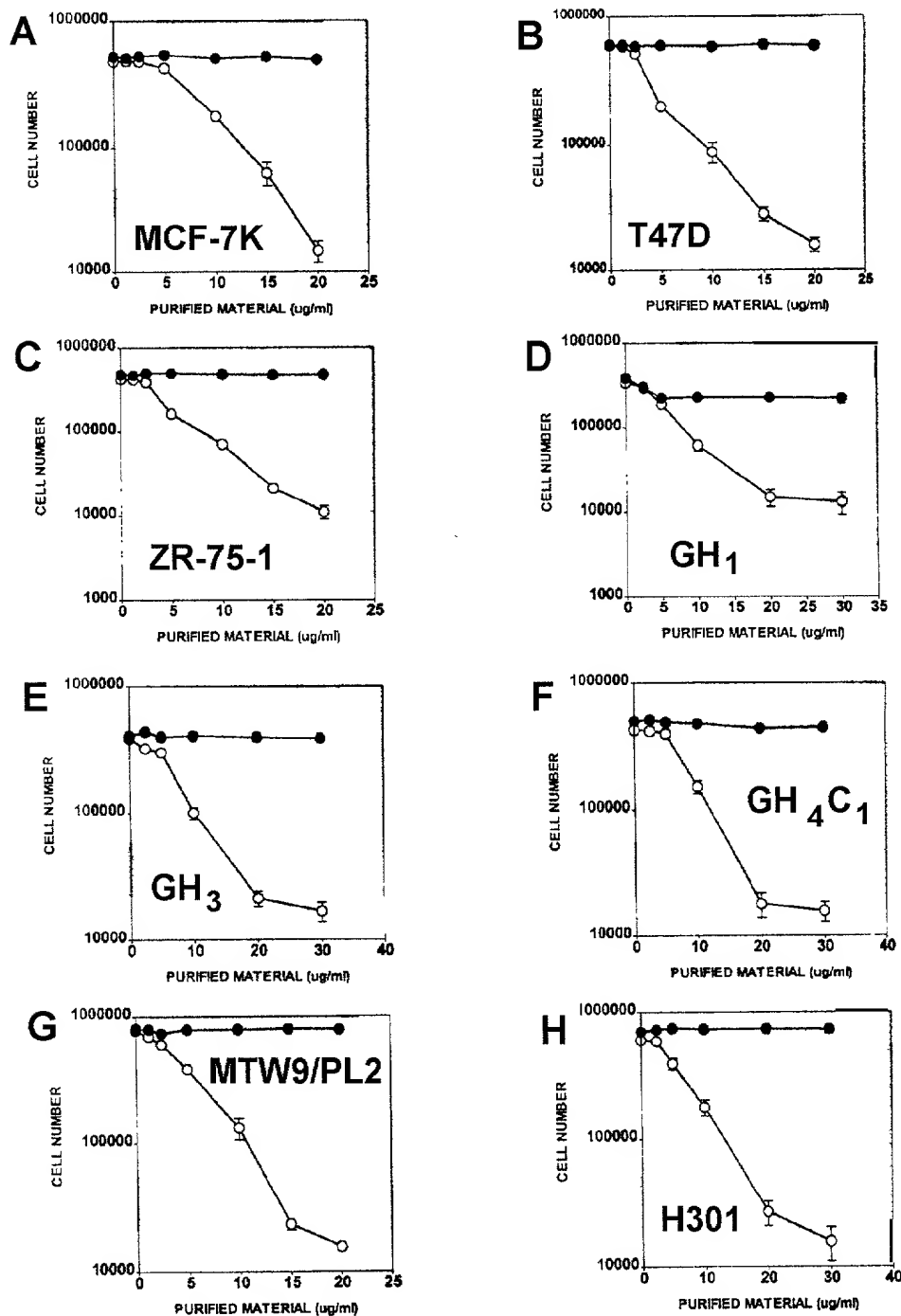
**B =  
 MTW9/PL2 IN DDM-2A**



**C =  
 GH<sub>4</sub>C<sub>1</sub> IN PCM 9**

**FIGURE 57**

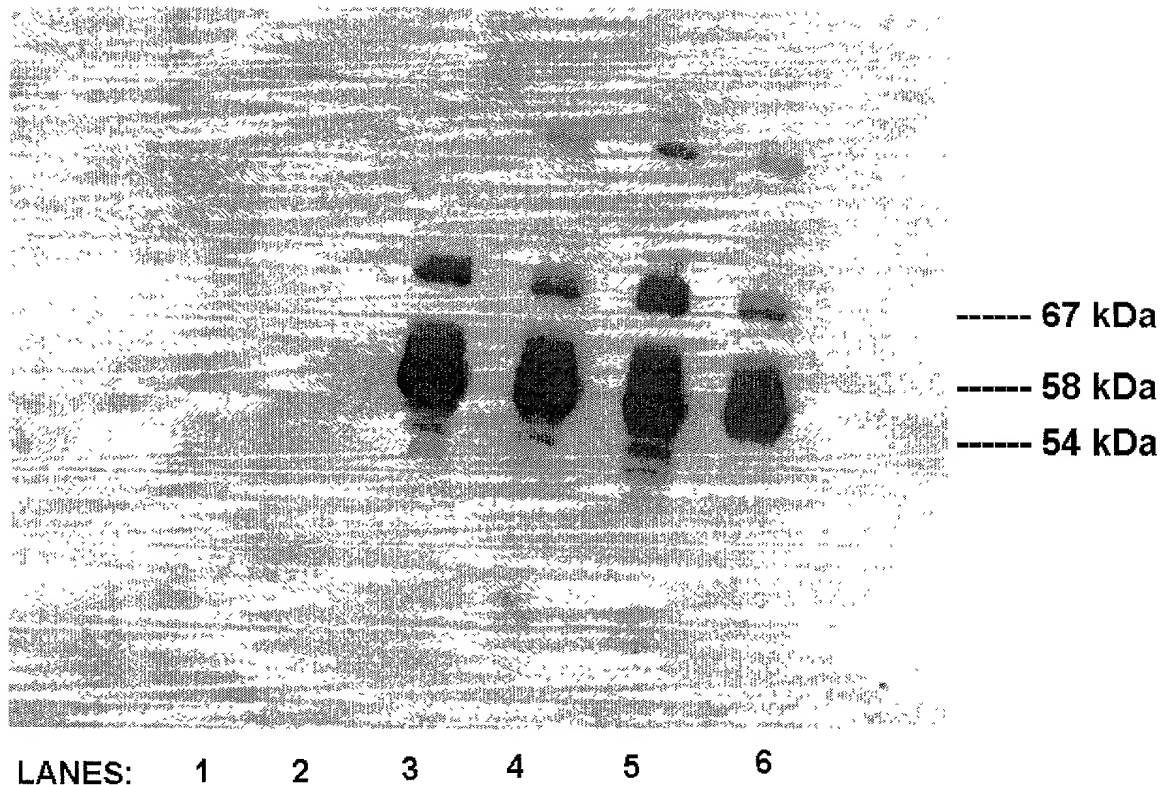
**EFFECT OF CA-PS-POOL II ON ESTROGEN  
 RESPONSIVE GROWTH IN SERUM FREE MEDIUM**



**LEGEND:** Open circles = - E<sub>2</sub>  
 Closed circles = + E<sub>2</sub>

## FIGURE 58

### WESTERN ANALYSIS OF CBG (POOL I) AND SHBG (POOL II) PREPARATION WITH ANTI-54 kDa



1 = CBG PREPARATION #5

2 = CBG PREPARATION #6

3 = SHBG PREPARATION #5.1

4 = SHBG PREPARATION #5.2

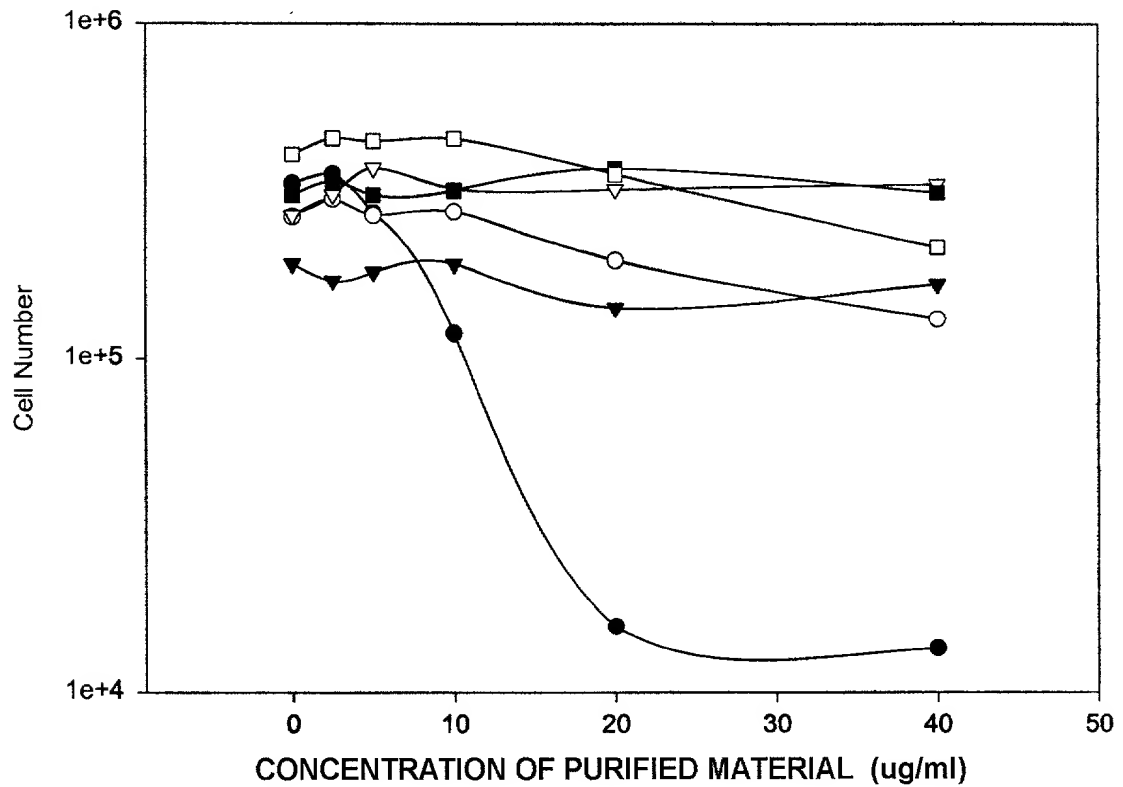
5 = SHBG PREPARATION #6.1

6 = SHBG PREPARATION #6.2

ANTIBODY = RABBIT ANTI-54 kDa 1:5000 DILUTION

**FIGURE 59**

**EFFECT OF ANTI-54kDa ANTISERUM ON MTW9/PL2  
CELLS GROWN IN THE PRESENCE OF CA-PS-POOL II**



**LEGEND:**

- No antibody
- Antibody 1:5000
- ▼ Antibody 1:1000
- ▽ Antibody 1:500
- Antibody 1:100
- Antibody 1:50

## FIGURE 60

### WESTERN BLOT OF COMMERCIAL PREPARATIONS OF HORSE IgA, IgG AND IgM WITH THE ANTI-54 kDa ANTIBODY

MkDa

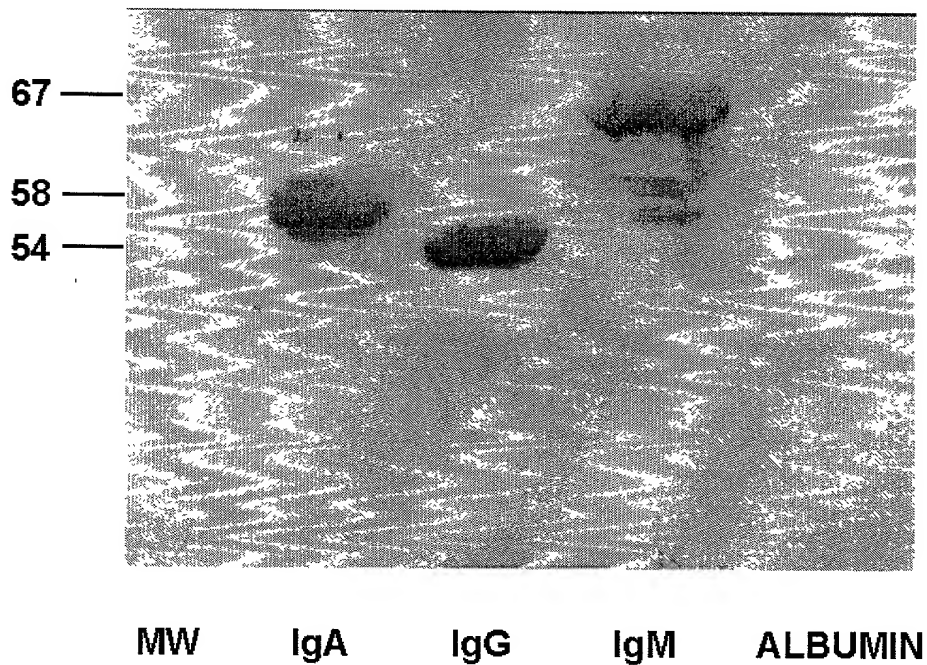
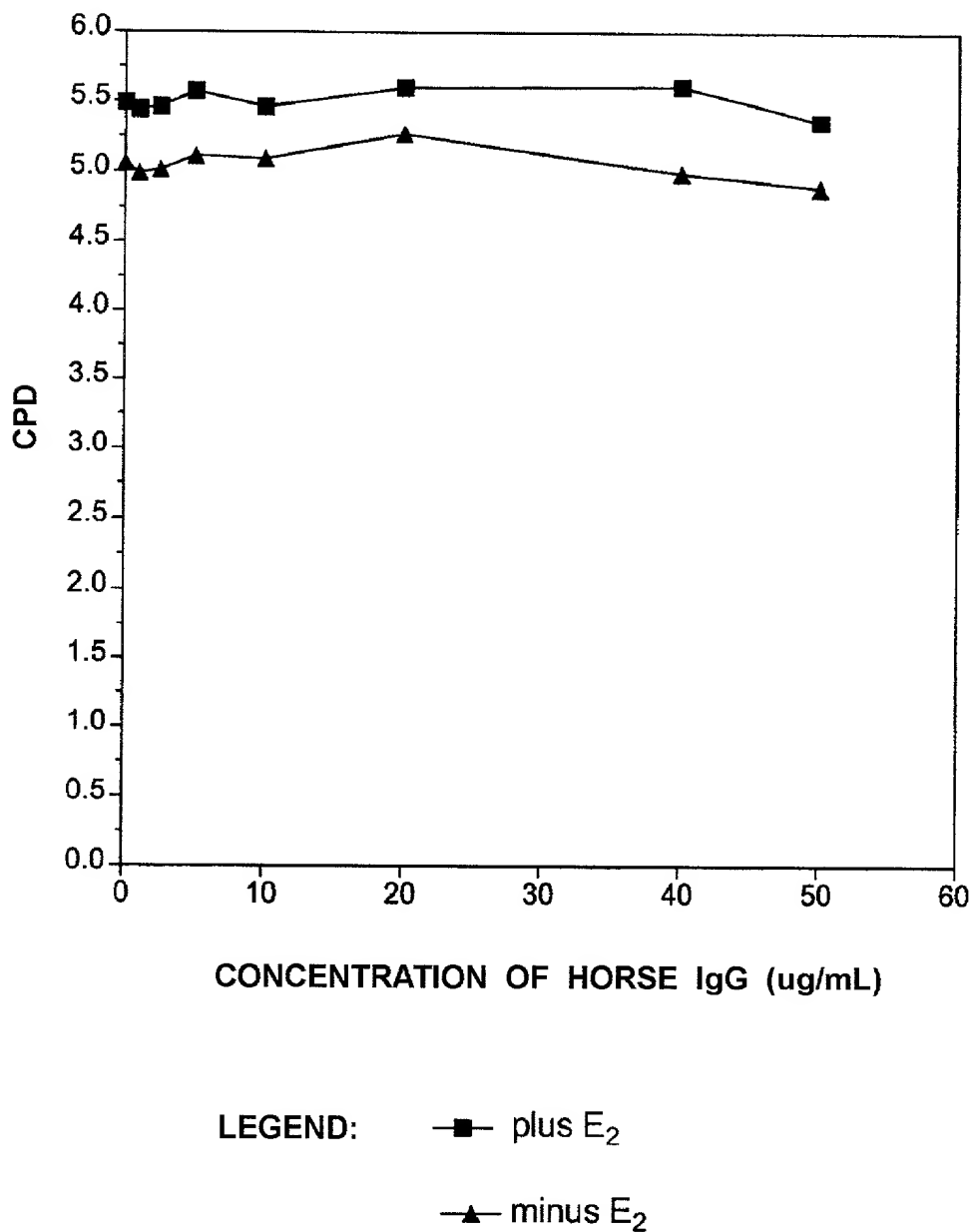


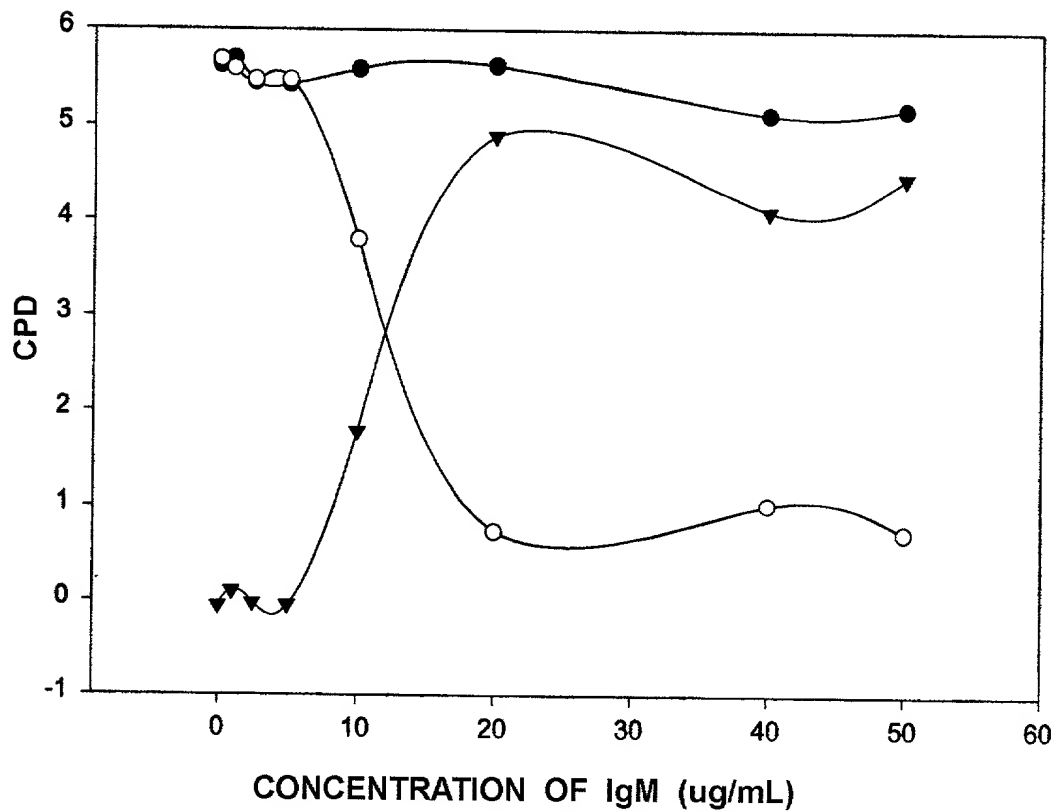
FIGURE 61

EFFECT OF COMMERCIALLY PURIFIED HORSE IgG  
ON MTW9/PL2 CELL GROWTH IN 2.5% CDE-HORSE SERUM



**FIGURE 62**

**EFFECT OF HORSE IgM ON GROWTH OF THE  
MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM  $\pm E_2$**

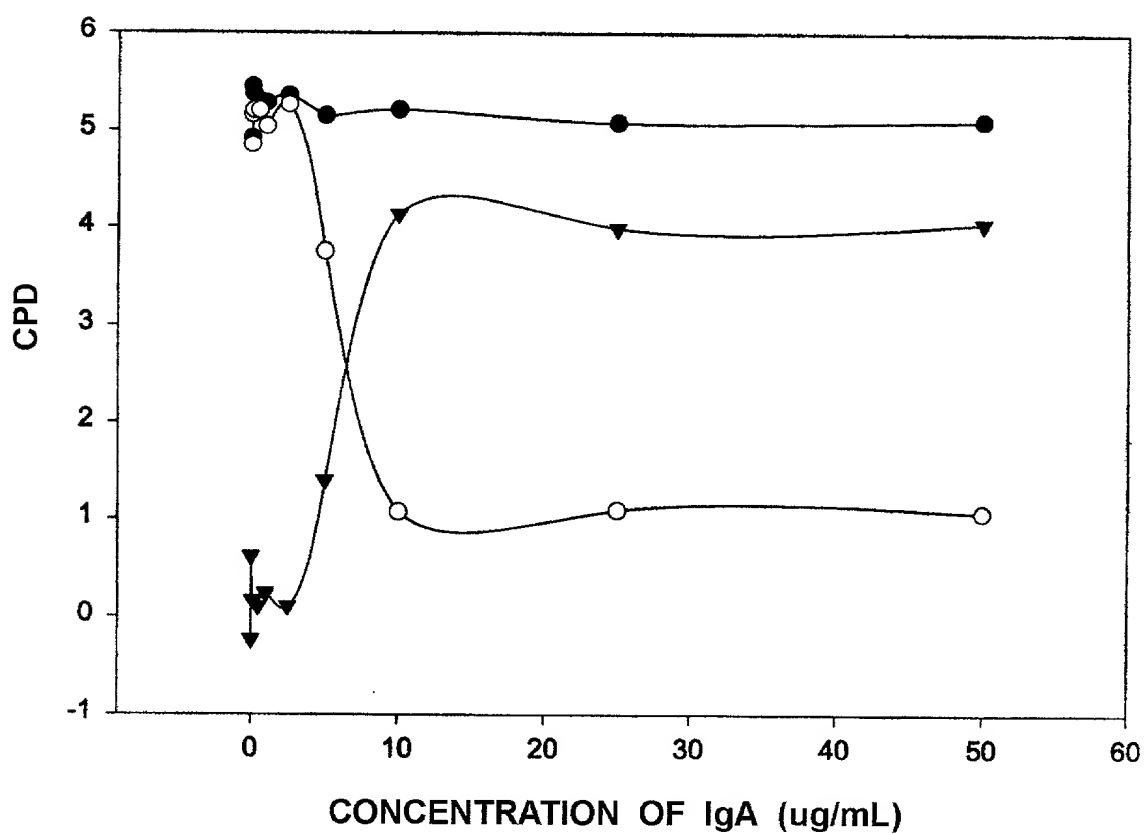


**LEGEND:**

- =  $+ E_2$
- =  $- E_2$
- ▼ = Estrogenic effect

**FIGURE 63**

**EFFECT OF HORSE IgA ON GROWTH OF THE  
 MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM  $\pm E_2$**

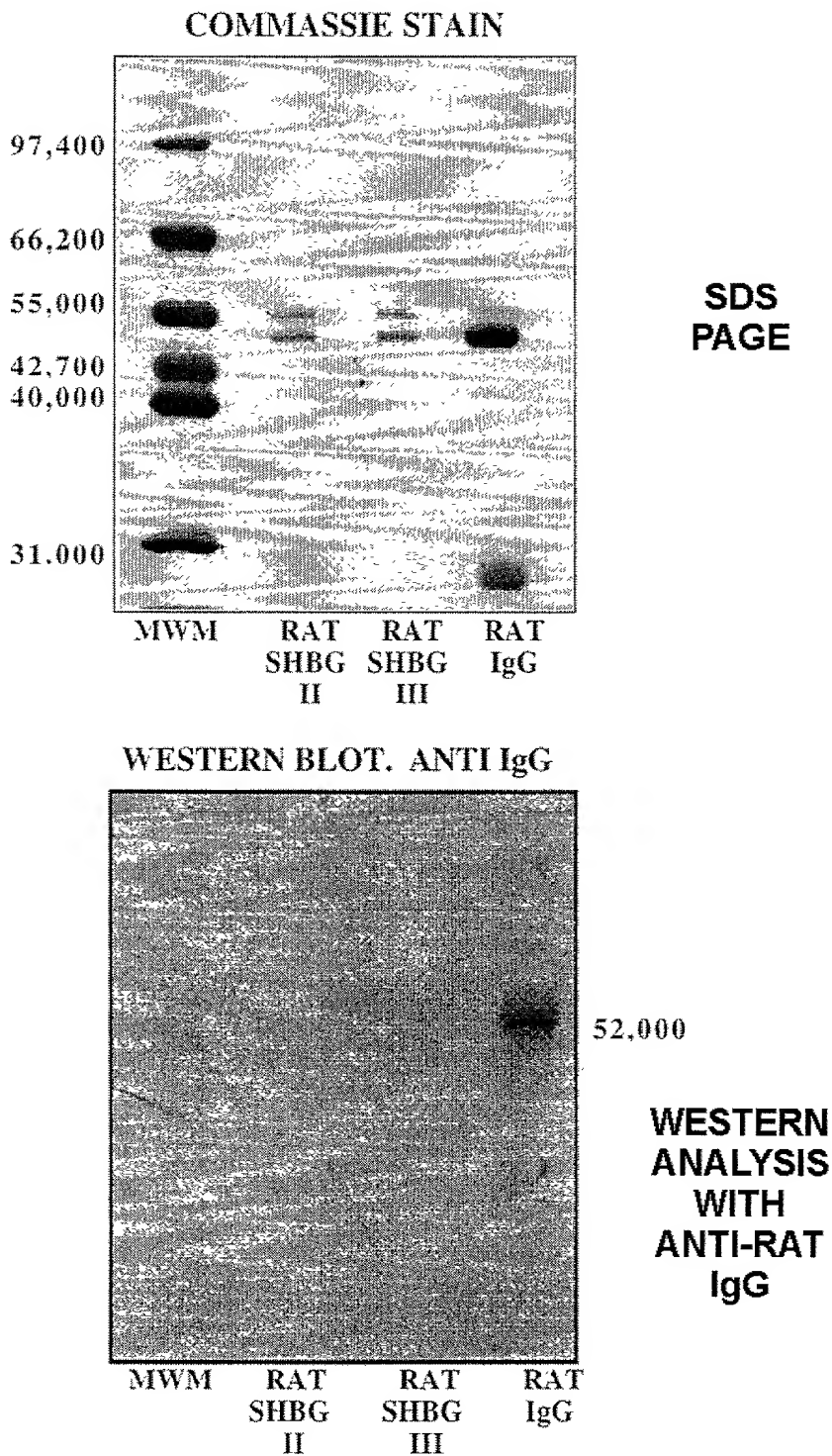


**LEGEND:**

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▼ = Estrogenic effect

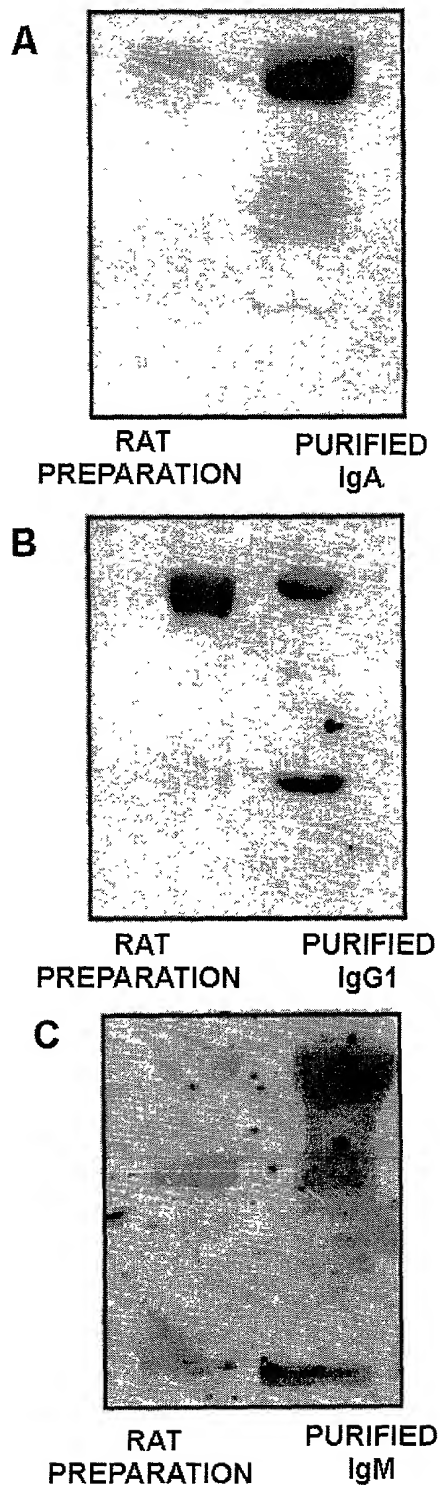
**FIGURE 64**

**SDS PAGE AND WESTERN ANALYSIS OF RAT  
"SHBG-LIKE" PREPARATIONS**



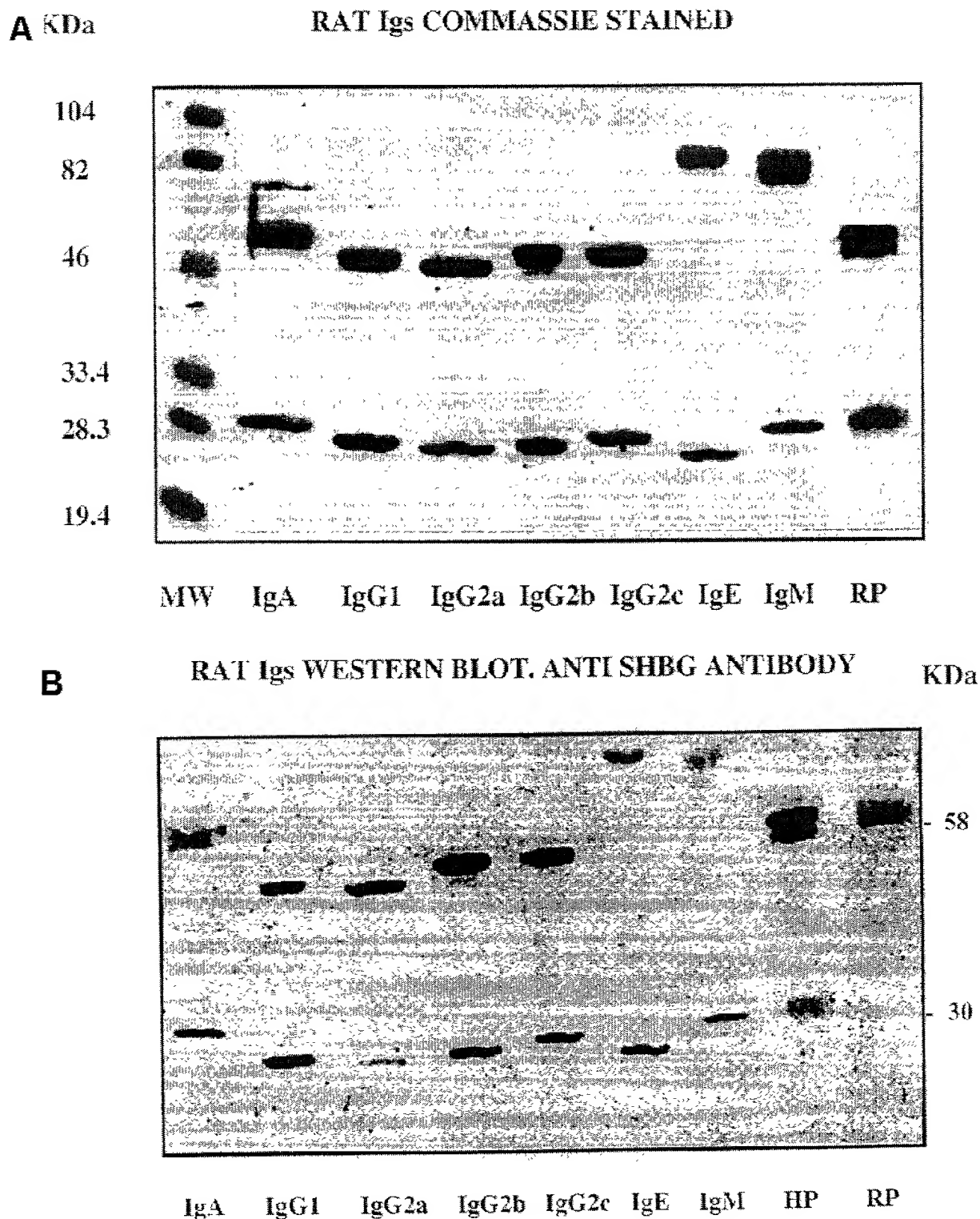
**FIGURE 65**

**CROSSREACTION OF THE PURIFIED RAT "SHBG-LIKE" PROTEINS  
WITH ANTI- IgA, IgG1 AND IgM MONOCLONAL ANTIBODIES**



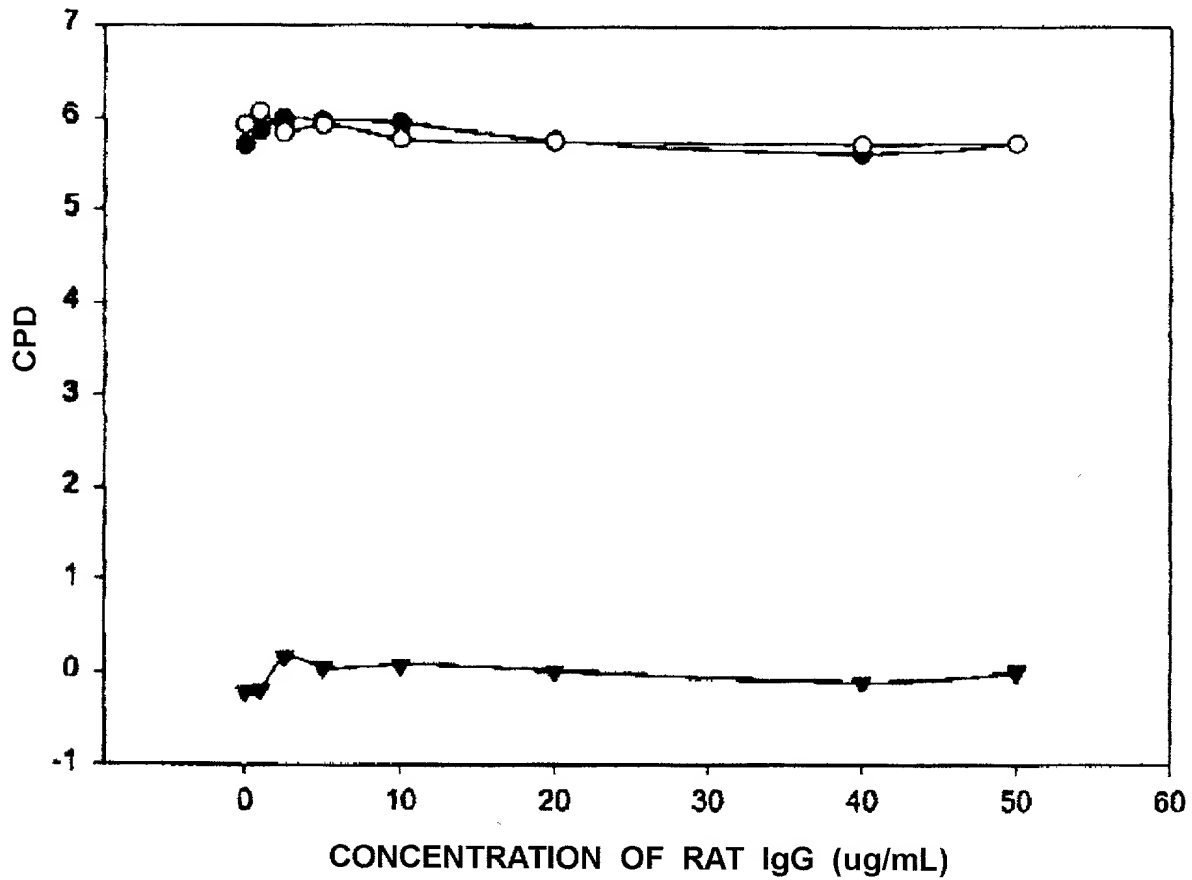
**FIGURE 66**

**SDS PAGE (A) AND WESTERN ANALYSIS (B)  
WITH ANTI-SHBG AND RAT Ig'S**



**FIGURE 67**

**EFFECT OF RAT IgG ON MTW9/PL2 CELL  
GROWTH IN 2.5% CDE RAT SERUM**



**LEGEND:**

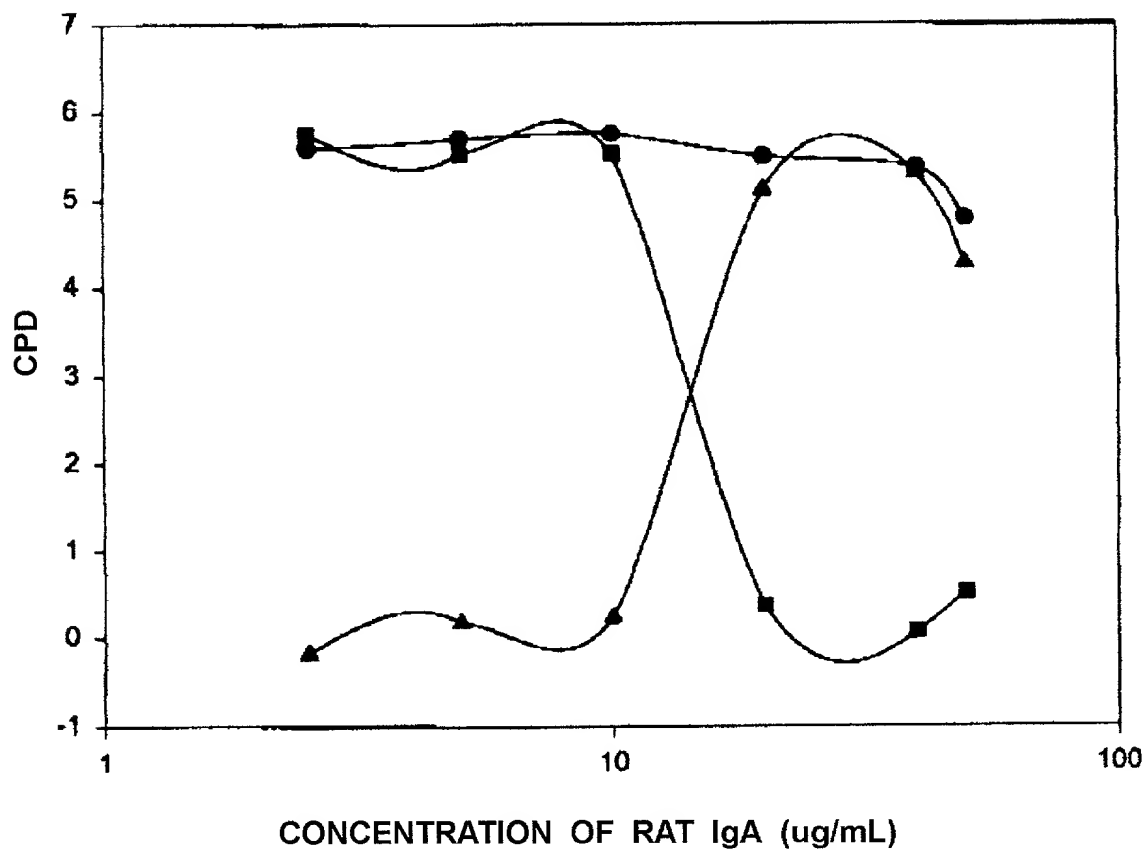
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

**FIGURE 68**

**EFFECT OF RAT IgA ON MTW9/PL2 CELL  
GROWTH IN 2.5% CDE RAT SERUM**



**LEGEND:**

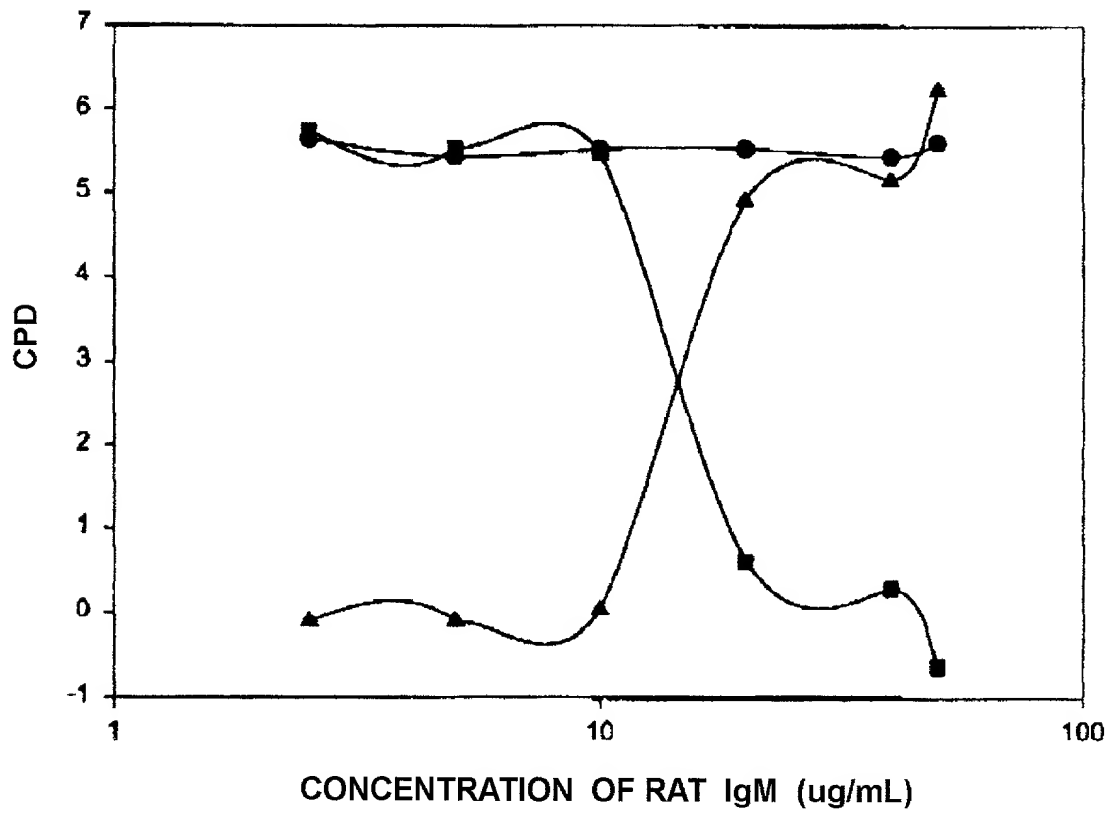
Closed circles = + E<sub>2</sub>

Closed squares = - E<sub>2</sub>

Closed triangles = Estrogenic effect

**FIGURE 69**

**EFFECT OF RAT IgM ON MTW9/PL2 CELL  
GROWTH IN 2.5% CDE RAT SERUM**



**LEGEND:**

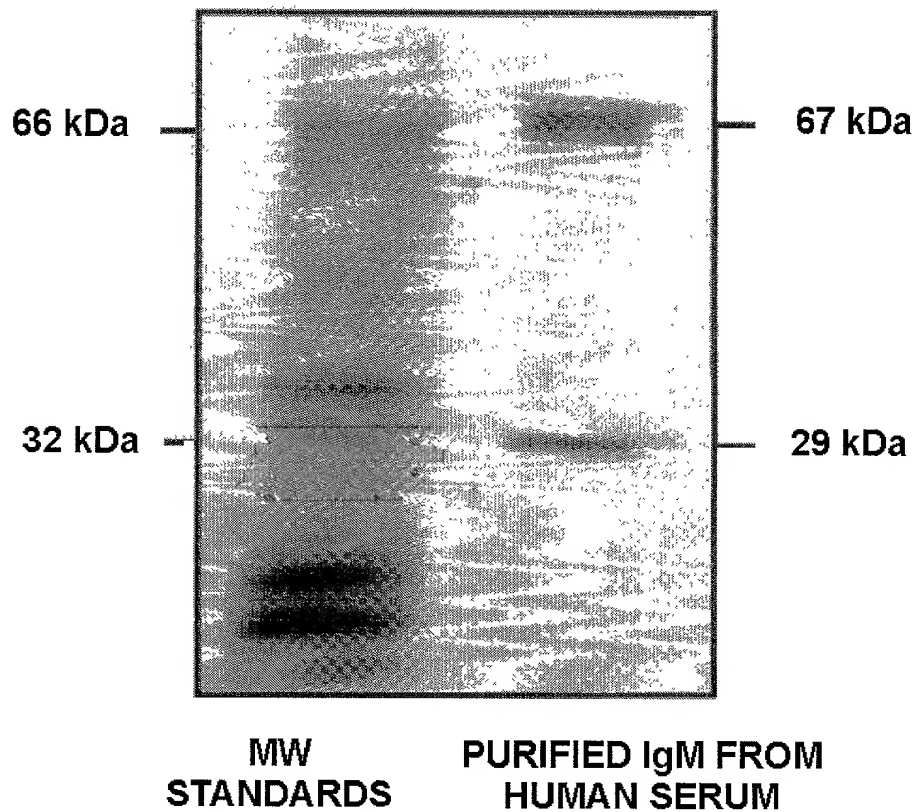
Closed squares = - E<sub>2</sub>

Closed circles = + E<sub>2</sub>

Closed triangles = Estrogenic effect

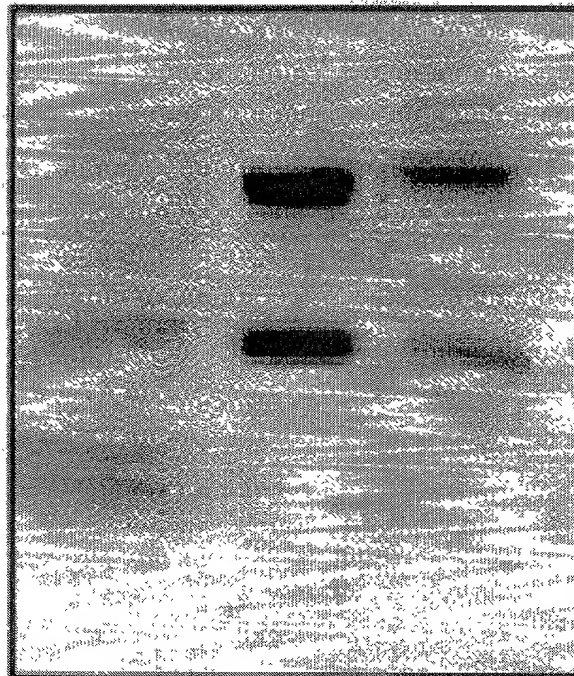
## FIGURE 70

### ELUTION OF IgM FROM MANNAN BINDING PROTEIN COLUMN



## FIGURE 71

### IgM PURIFICATION FROM PLASMA BY JACALIN



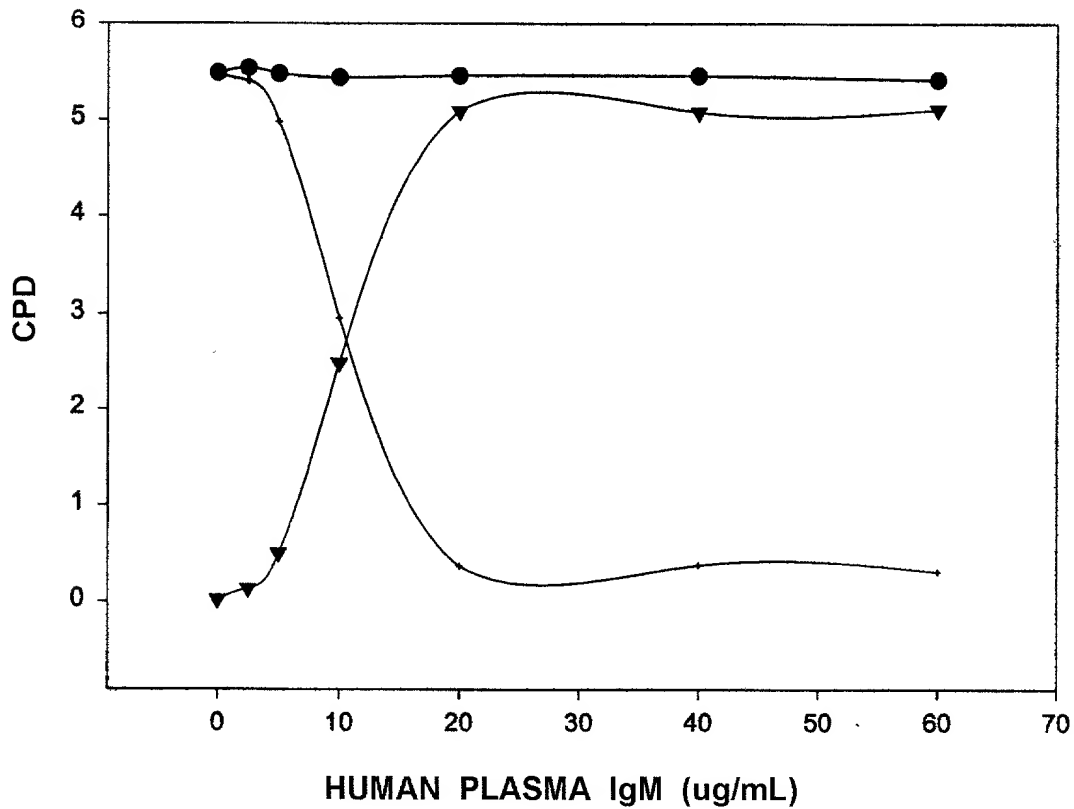
MW

HUMAN  
IgA

PURIFIED  
IgA

FIGURE 72

EFFECT OF IgM ISOLATED FROM HUMAN PLASMA  
ON MTW9/PL2 GROWTH IN SERUM-FREE CONDITIONS

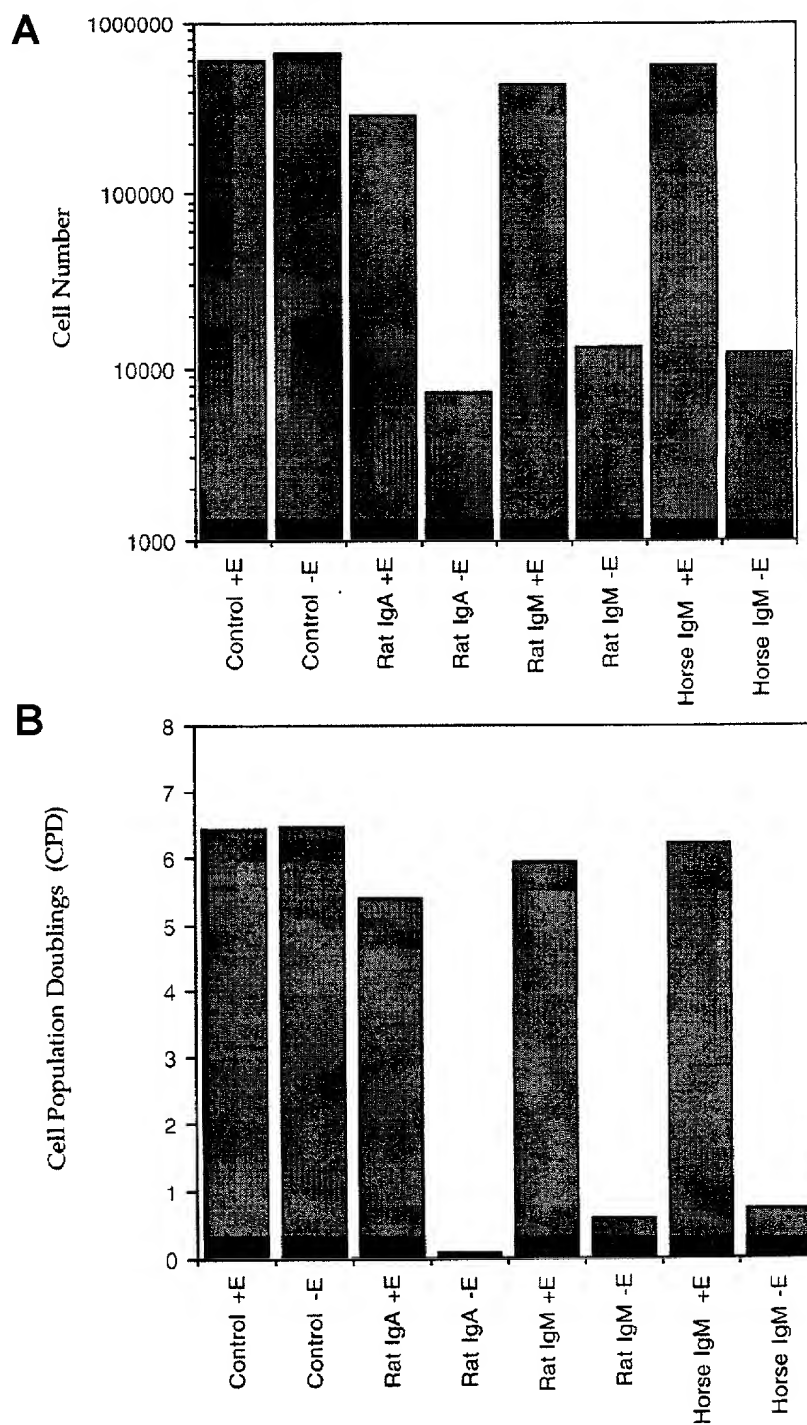


LEGEND:

- = + E<sub>2</sub>
- △— = - E<sub>2</sub>
- ▼— = Estrogenic effect

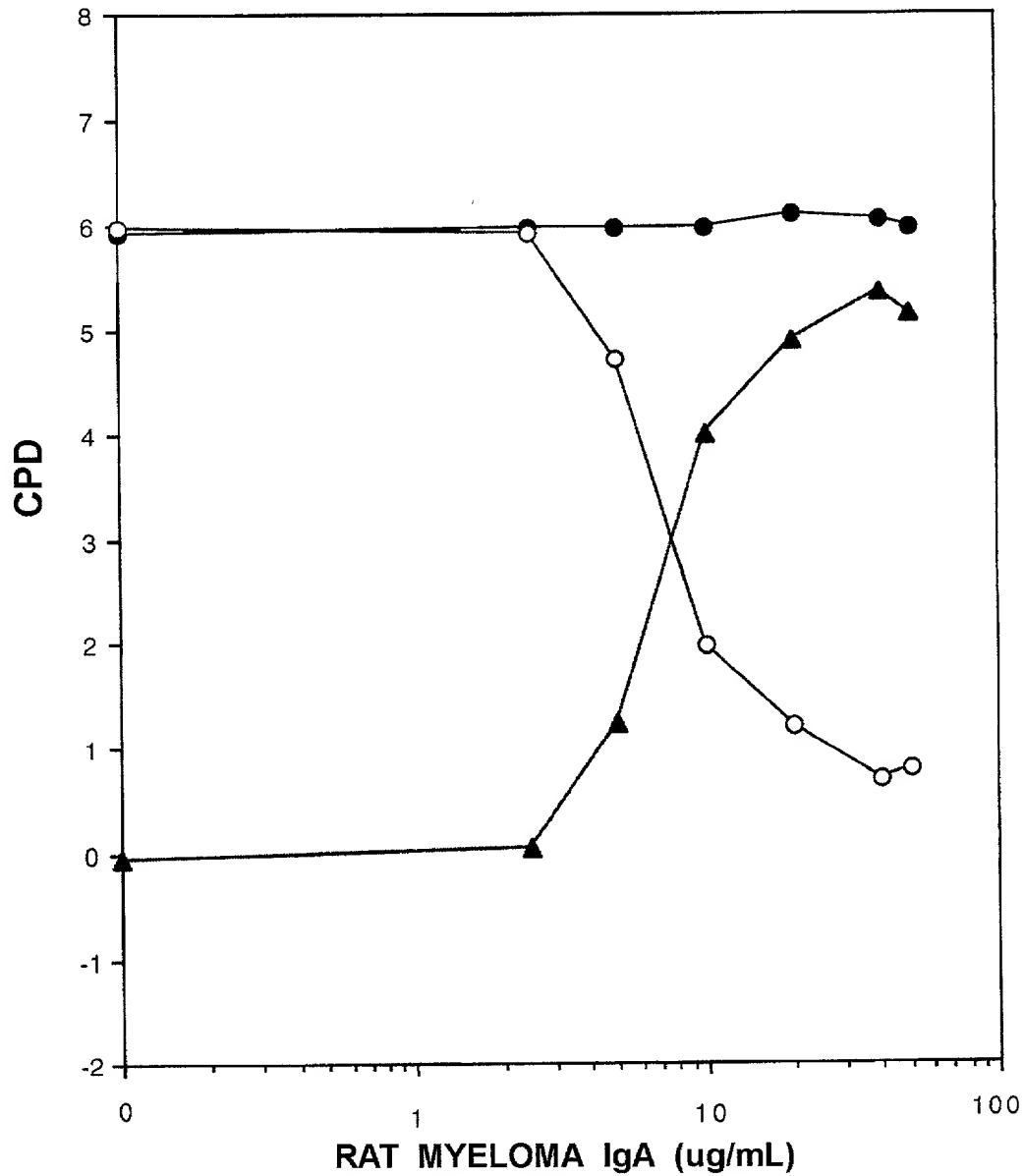
**FIGURE 73**

**THE EFFECT OF VARIOUS IgA AND IgM PREPARATIONS  
ON MTW9/PL2 CELLS GROWN IN SERUM-FREE MEDIUM**



**FIGURE 74**

**RAT MYELOMA IgA TITRATION ON GH<sub>1</sub> CELLS  
 GROWN IN SERUM-FREE CONDITIONS**



**LEGEND:**

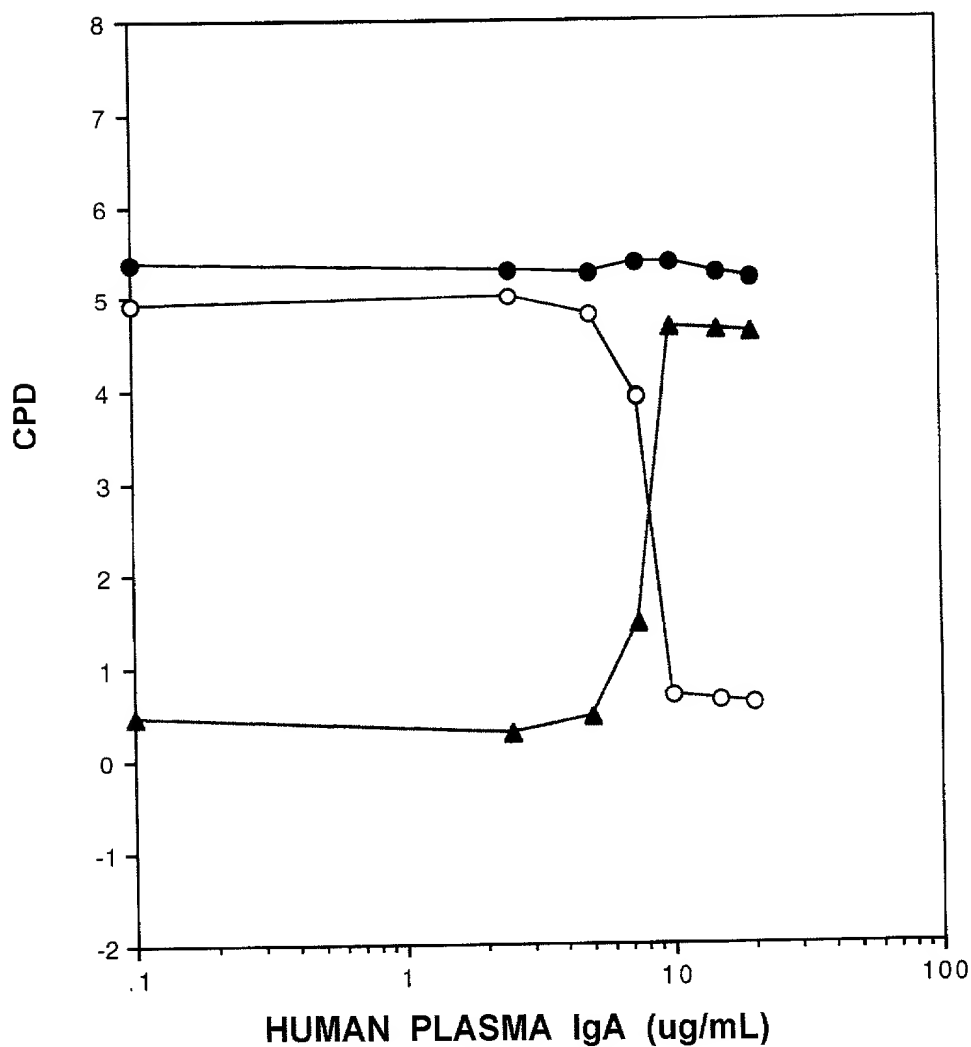
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

**FIGURE 75**

**HUMAN PLASMA IgA TITRATION ON GH<sub>1</sub> CELLS  
 GROWN IN SERUM-FREE CONDITIONS**



**LEGEND:**

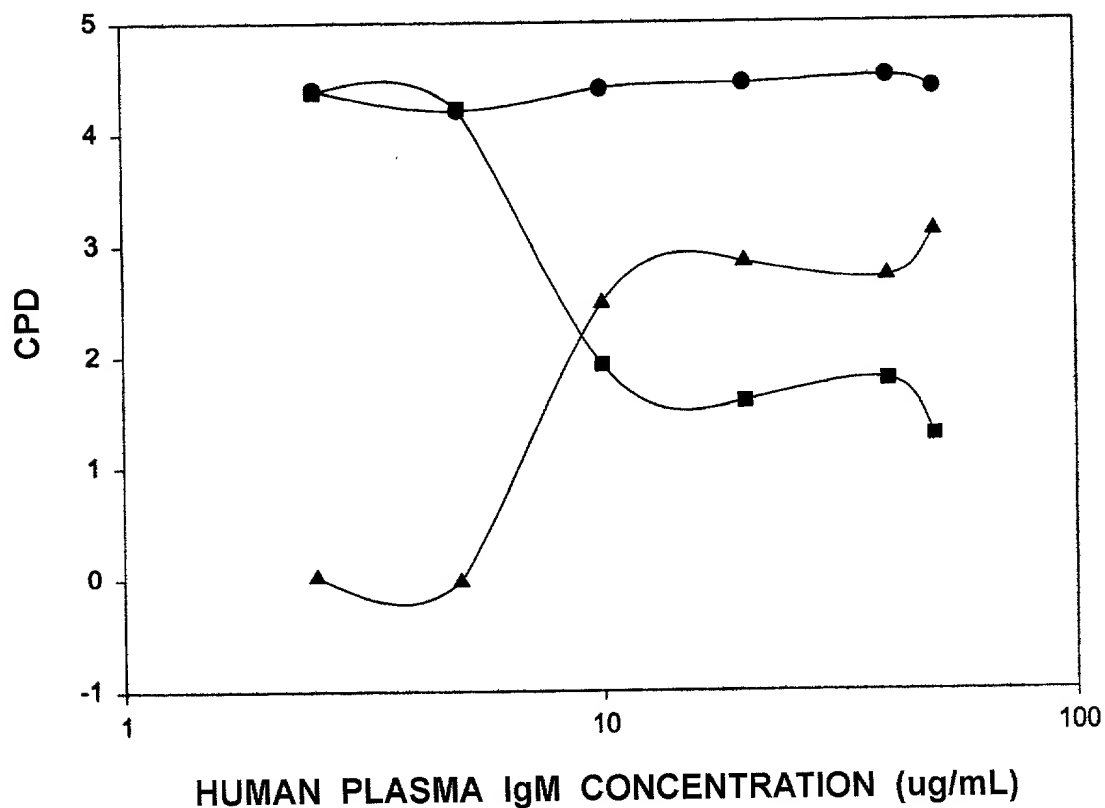
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

FIGURE 76

HUMAN PLASMA IgM TITRATION ON GH<sub>1</sub> CELLS  
GROWN IN SERUM-FREE CONDITIONS

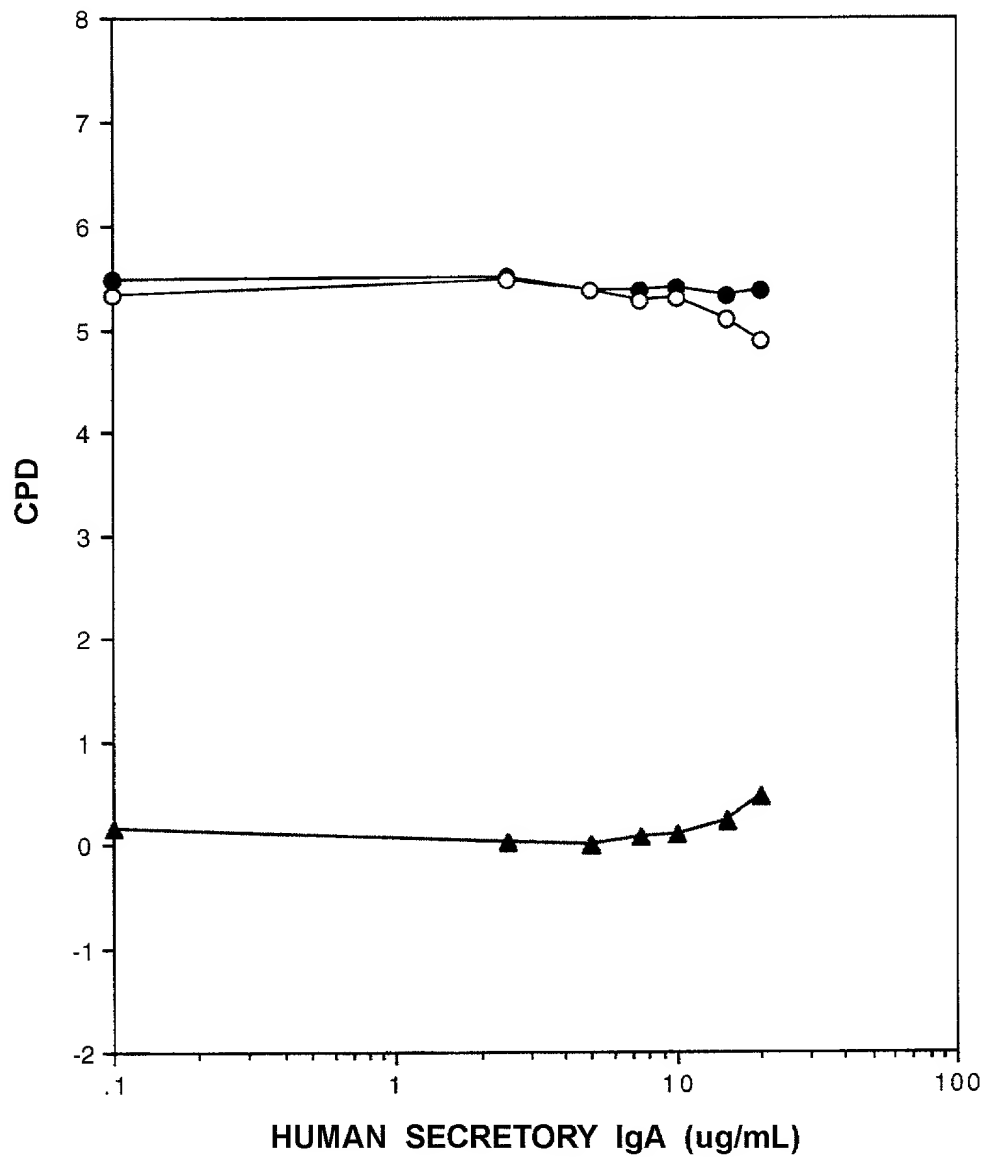


LEGEND:

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▲— = Estrogenic effect

**FIGURE 77**

**EFFECT OF HUMAN SECRETORY IgA ON  
GH<sub>1</sub> CELLS GROWN IN SERUM-FREE CONDITIONS**



**LEGEND:**

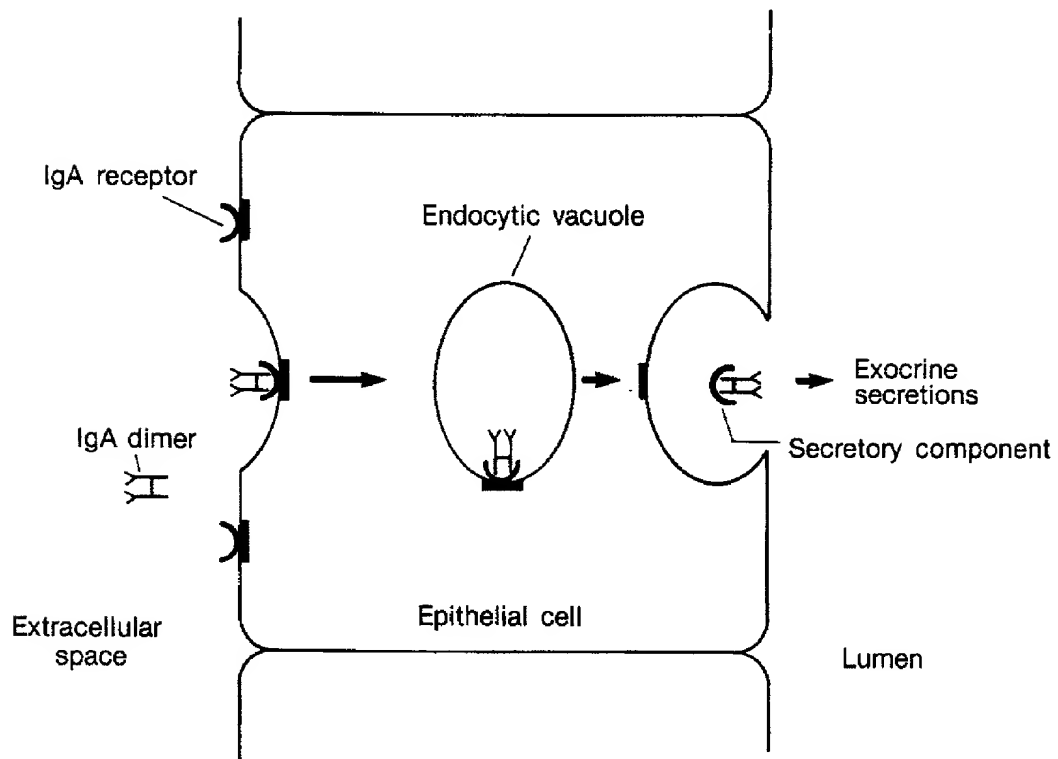
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

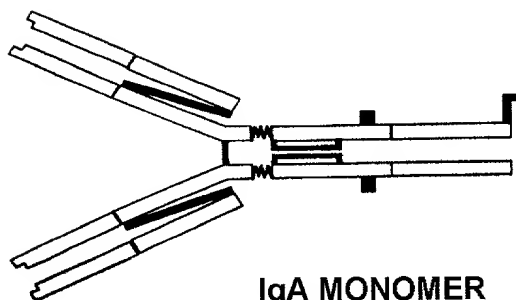
**FIGURE 78**

**MECHANISM OF TRANSCYTOSIS OF IgA AND IgM  
BY MUCOSAL EPITHELIAL CELLS**

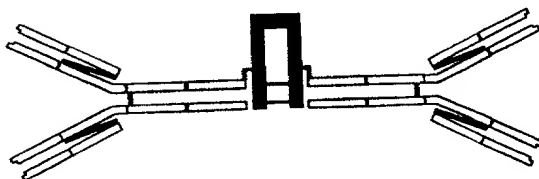


**FIGURE 79**

**ESSENTIAL STRUCTURES OF HUMAN  
PLASMA AND SECRETORY IgA**



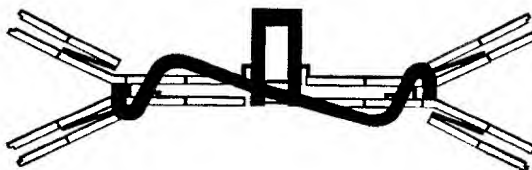
**J CHAIN**



**IgA DIMER WITH  
ATTACHED J CHAIN (ACTIVE)**



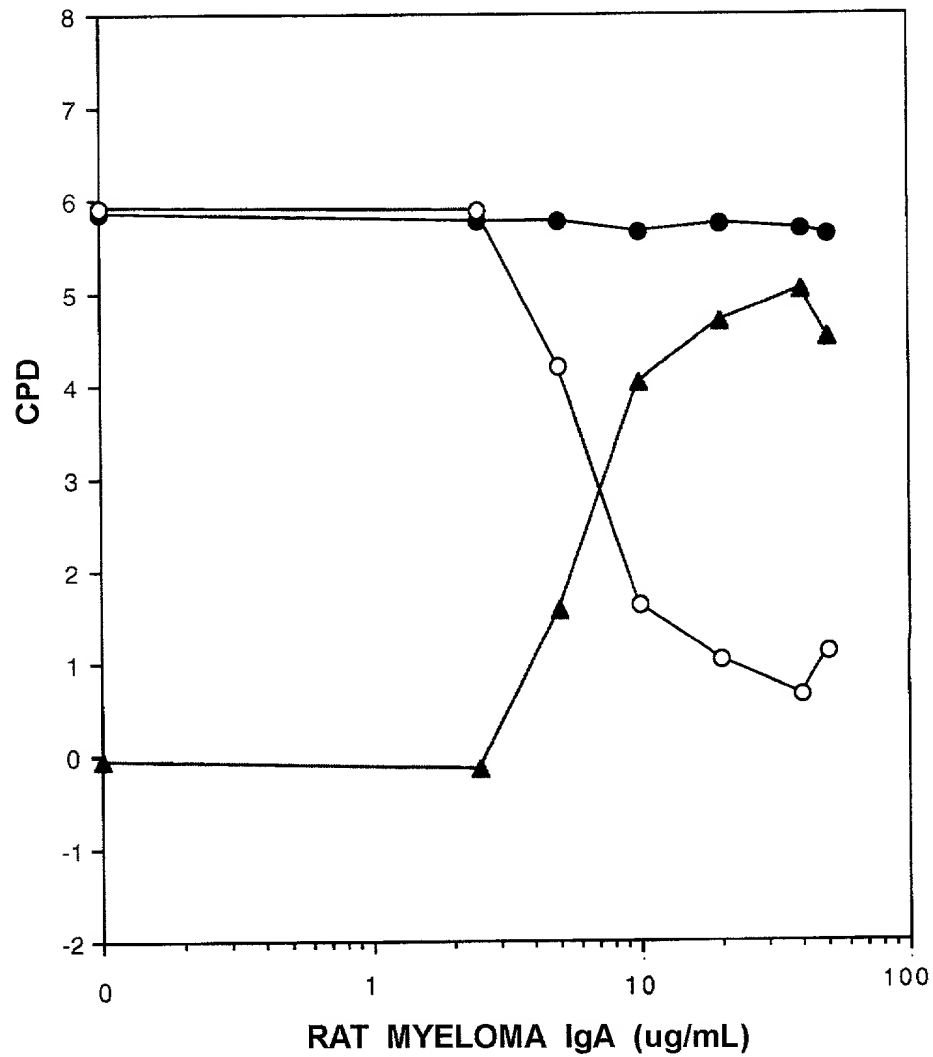
**SECRETORY PIECE OR  
SECRETORY COMPONENT  
(80% POLY-IgR)**



**SECRETORY IgA SHOWING J CHAIN  
AND SECRETORY COMPONENT (INACTIVE)**

**FIGURE 80**

**EFFECT OF RAT MYELOMA IgA ON GH<sub>3</sub>  
CELLS GROWN IN SERUM-FREE MEDIUM**



**LEGEND:**

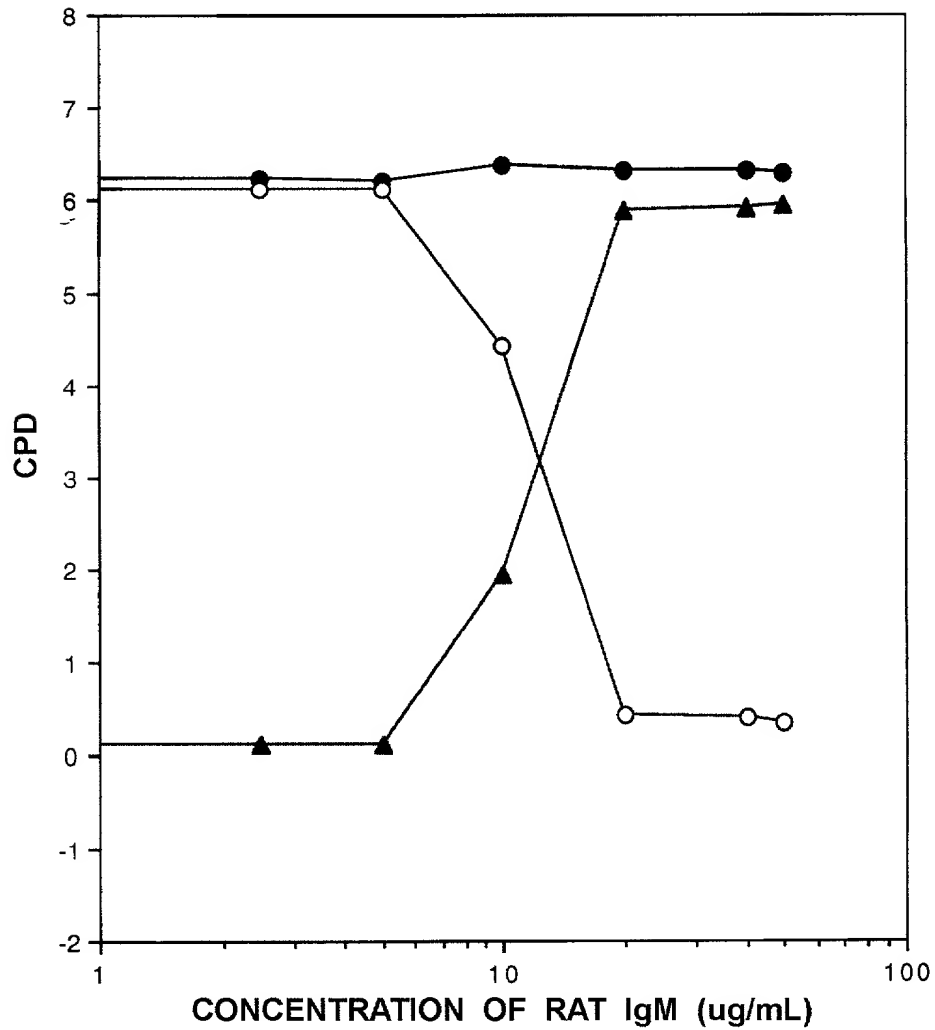
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

**FIGURE 81**

**EFFECT OF RAT IgM ON GH<sub>3</sub> CELL  
 GROWTH IN SERUM-FREE MEDIUM**

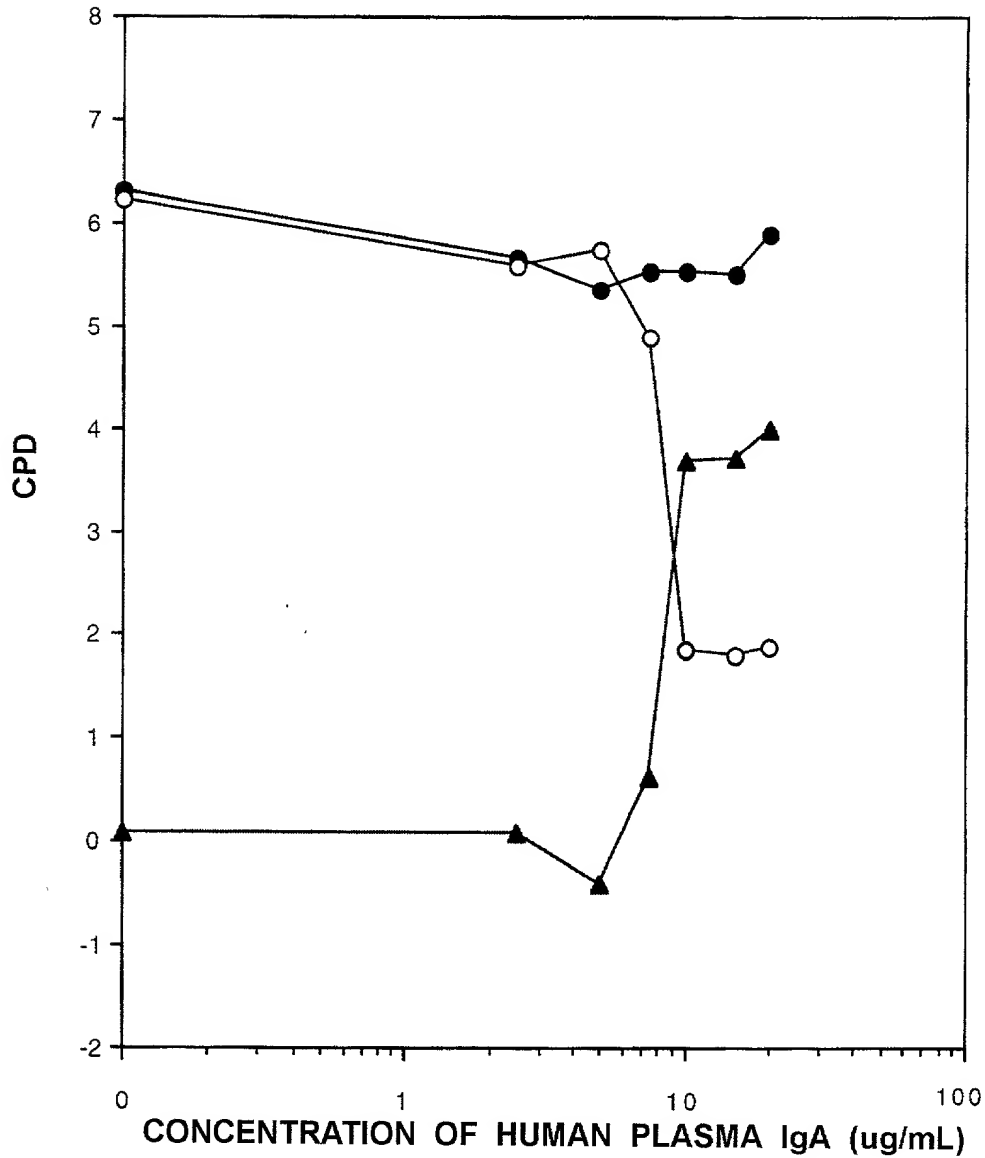


**LEGEND:**

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▲— = Estrogenic effect

**FIGURE 82**

**EFFECT OF HUMAN PLASMA IgA ON GH<sub>3</sub>  
 CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**

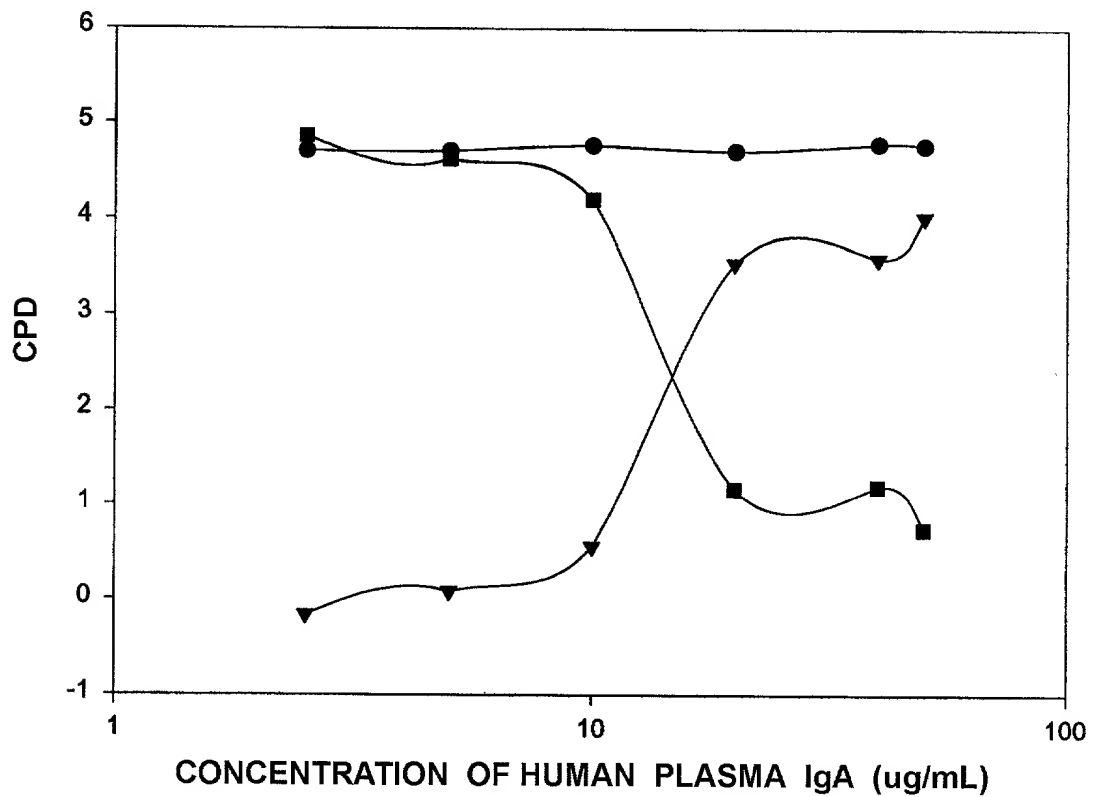
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

FIGURE 83

EFFECT OF HUMAN PLASMA IgM ON GH<sub>3</sub>  
CELL GROWTH IN SERUM-FREE MEDIUM

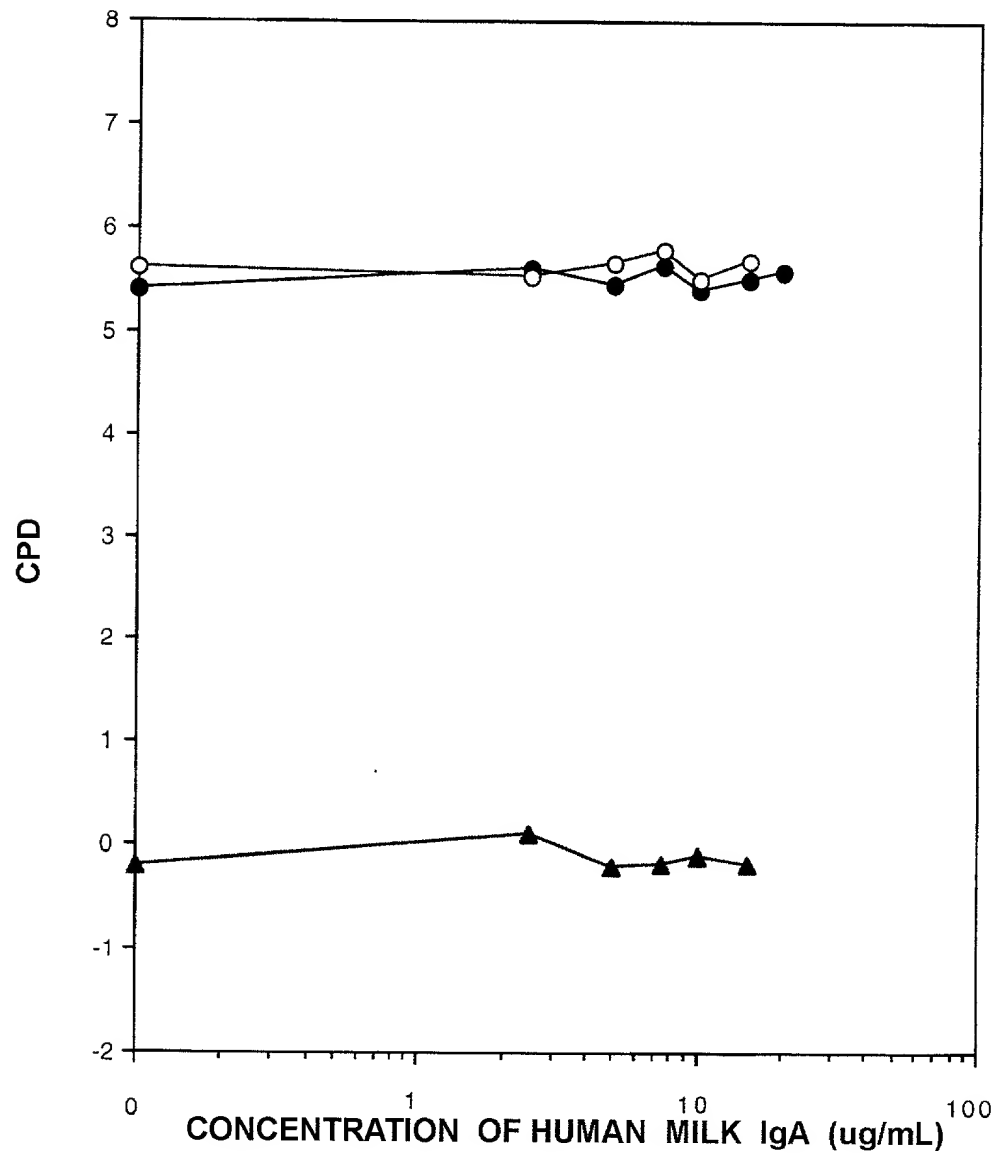


LEGEND:

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▼— = Estrogenic effect

**FIGURE 84**

**EFFECT OF HUMAN MILK SECRETORY IgA ON  
GH<sub>3</sub> CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**

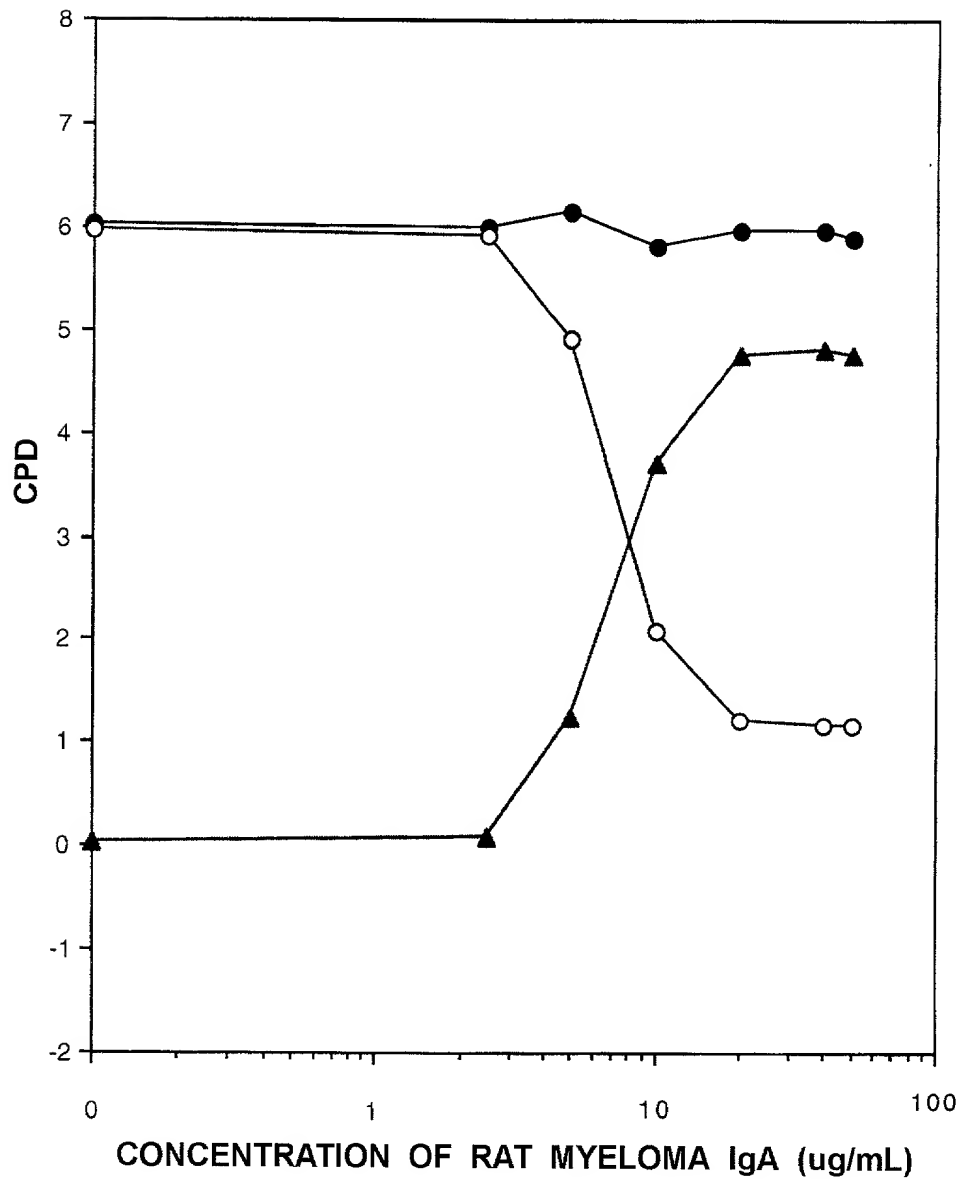
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

**FIGURE 85**

**EFFECT OF RAT MYELOMA IgA ON GH<sub>4</sub>  
CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**

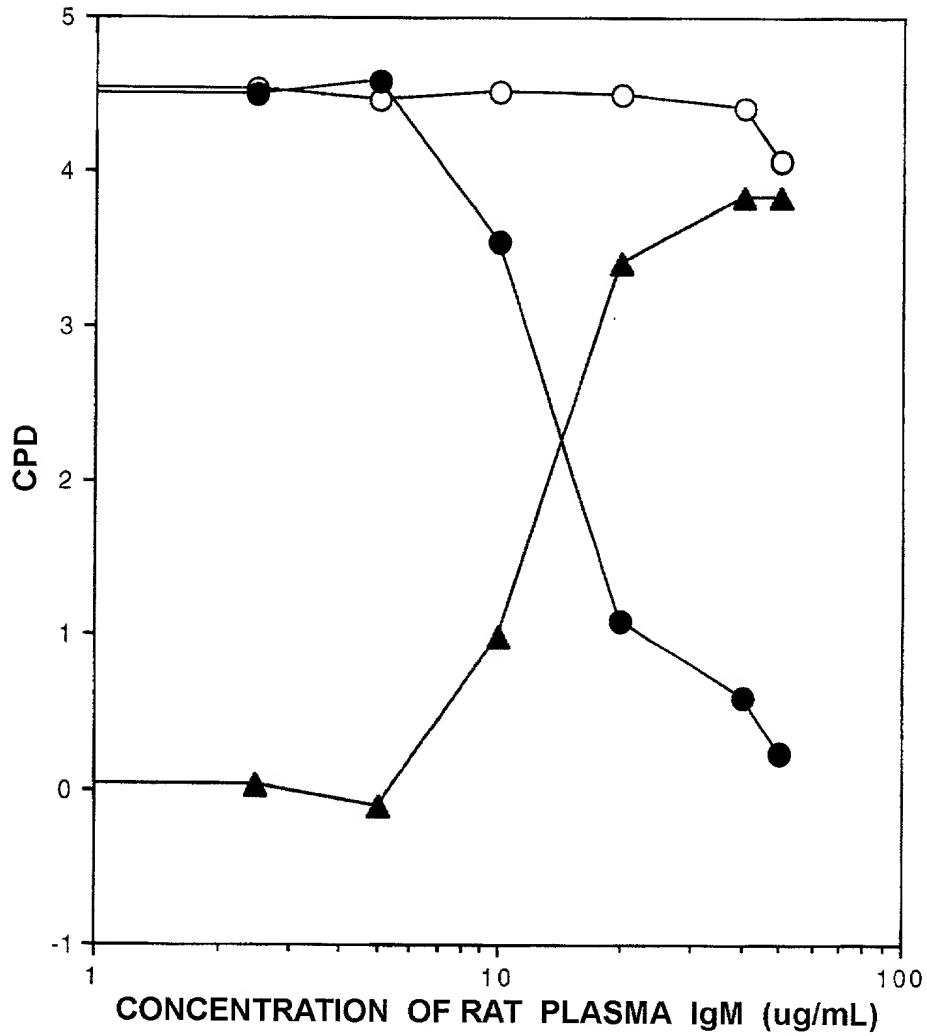
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

FIGURE 86

EFFECT OF RAT PLASMA IgM ON GH<sub>4</sub>  
CELL GROWTH IN SERUM-FREE MEDIUM

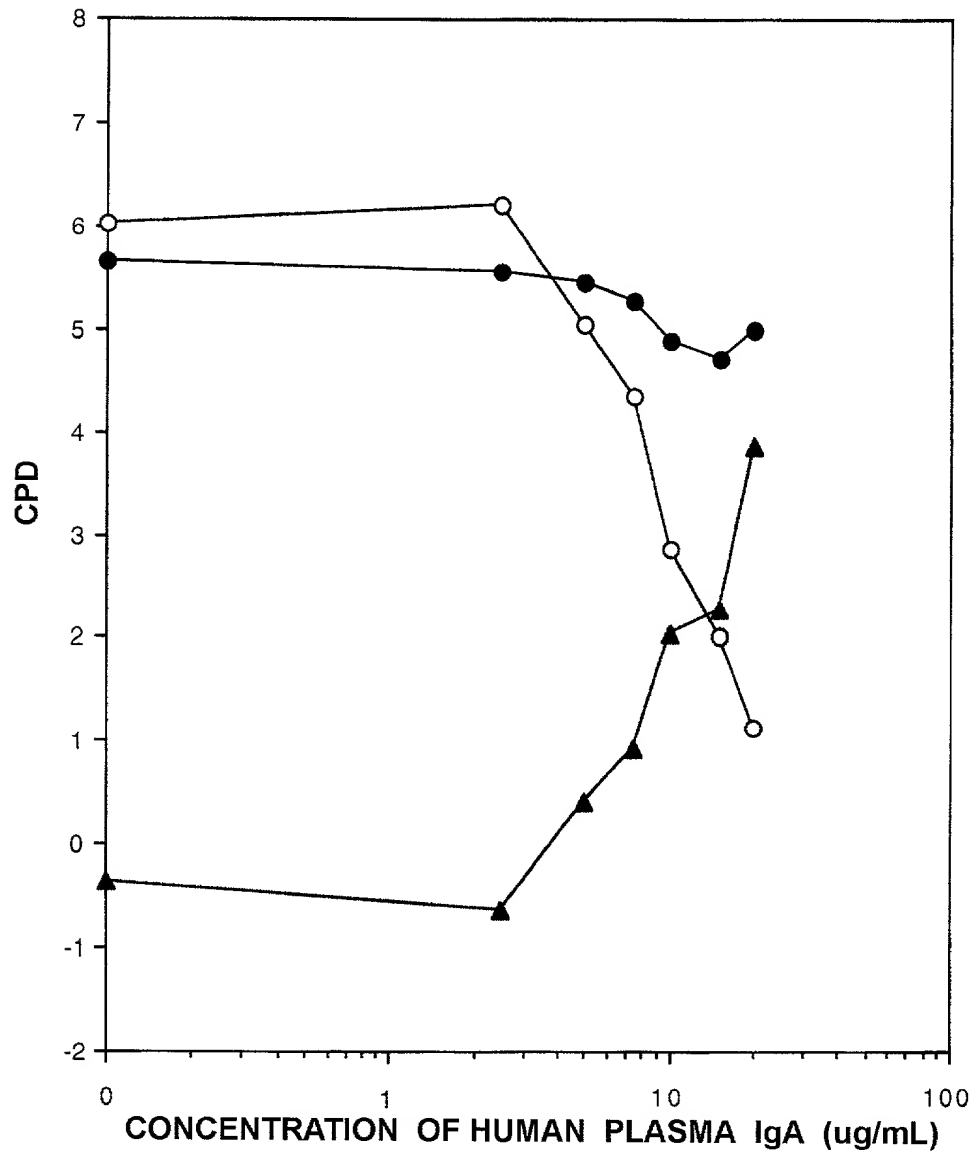


LEGEND:

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▲— = Estrogenic effect

**FIGURE 87**

**EFFECT OF HUMAN PLASMA IgA ON GH<sub>4</sub>C<sub>1</sub>  
 CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**

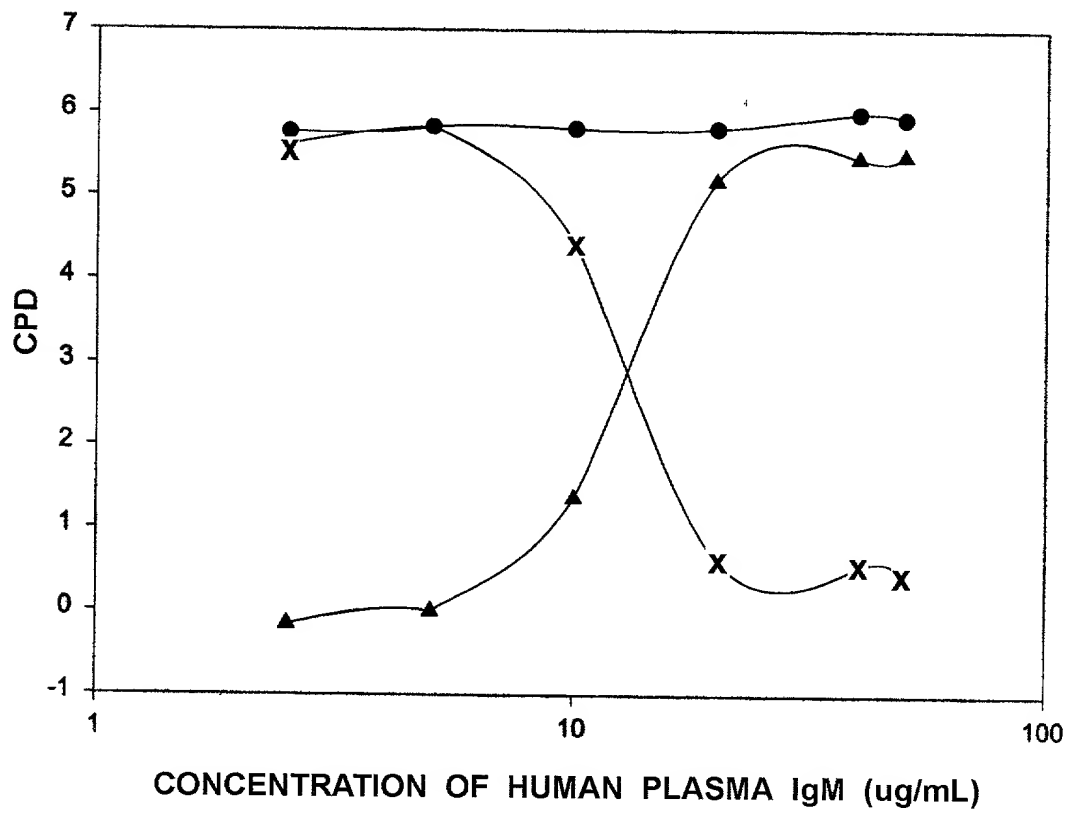
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

FIGURE 88

EFFECT OF HUMAN PLASMA IgM ON GH<sub>4</sub>C<sub>1</sub>  
CELL GROWTH IN SERUM-FREE MEDIUM

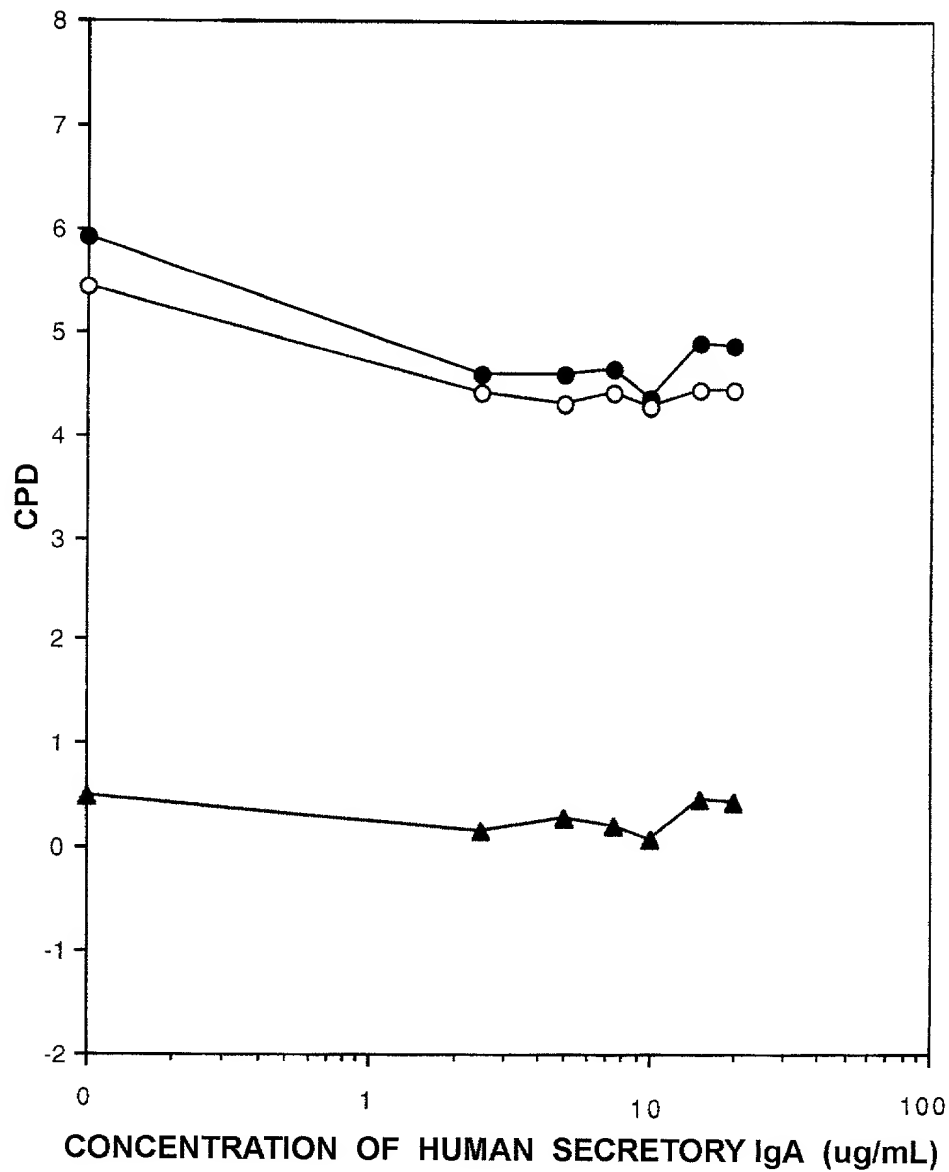


LEGEND:

- = + E<sub>2</sub>
- X— = - E<sub>2</sub>
- ▲— = Estrogenic effect

**FIGURE 89**

**EFFECT OF HUMAN MILK SECRETORY IgA ON  
 GH<sub>4</sub>C<sub>1</sub> CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**

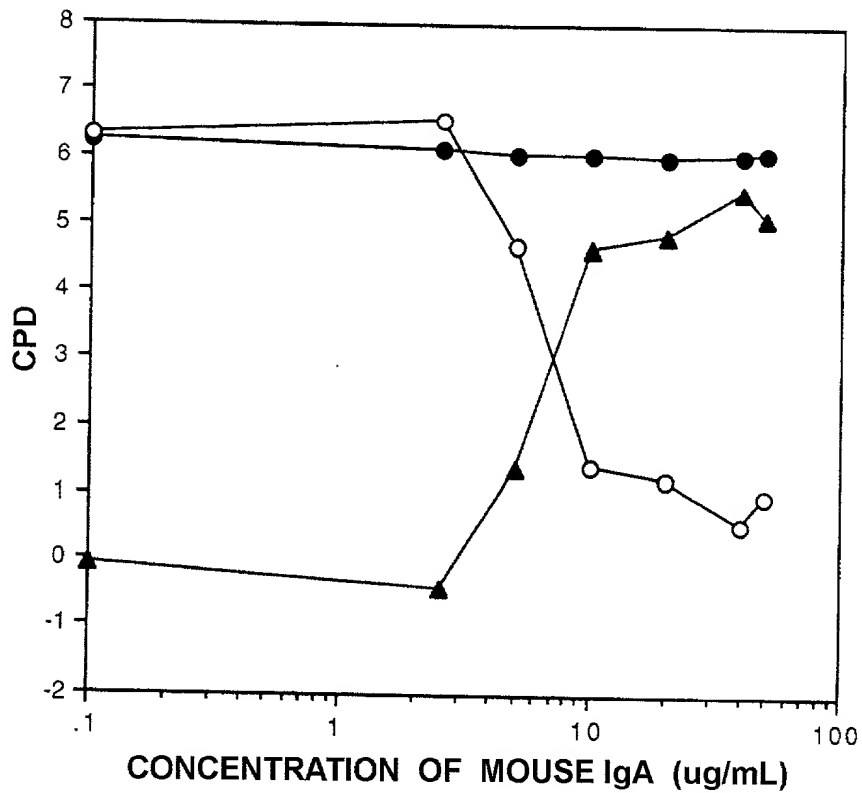
Closed circles = + E<sub>2</sub>

Open circles = - E<sub>2</sub>

Closed triangles = Estrogenic effect

**FIGURE 90**

**EFFECT OF MOUSE IgA ON H301 CELL  
GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**

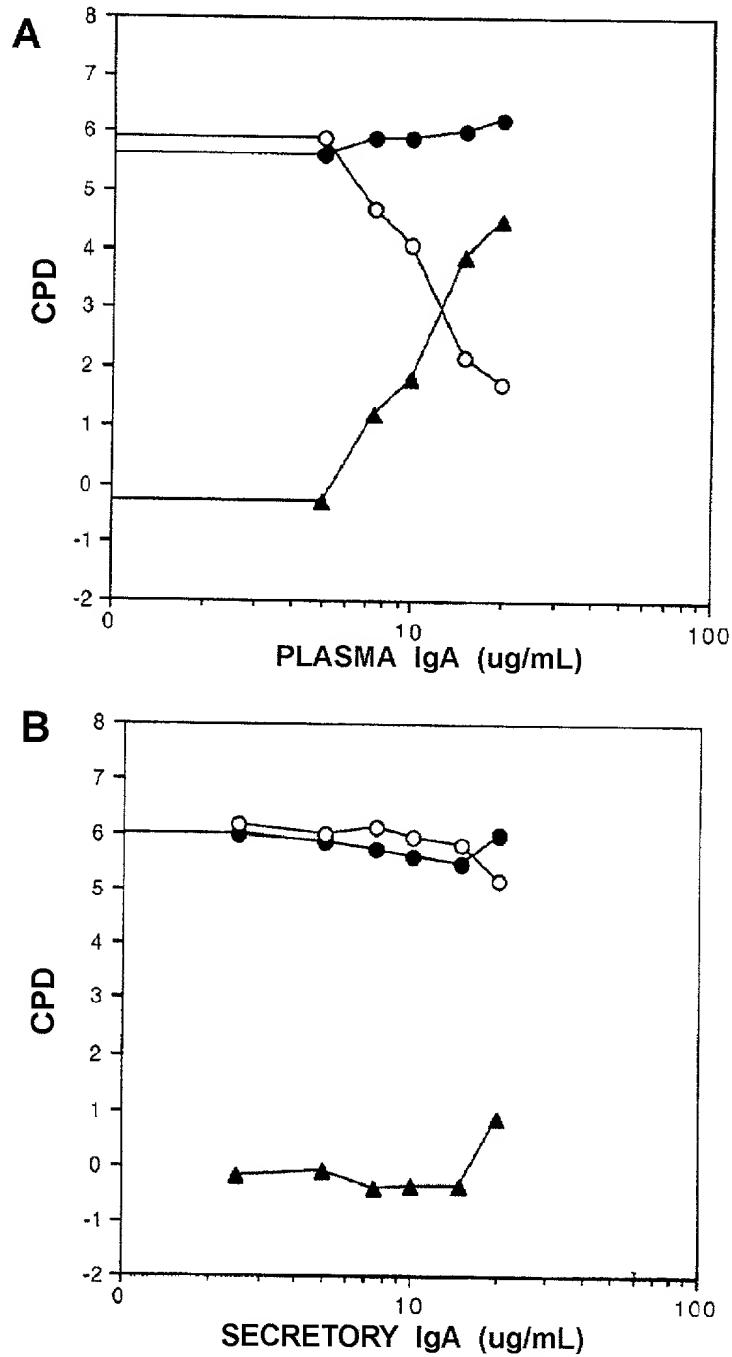
**Closed circles = + E<sub>2</sub>**

**Open circles = - E<sub>2</sub>**

**Closed triangles = Estrogenic effect**

**FIGURE 91**

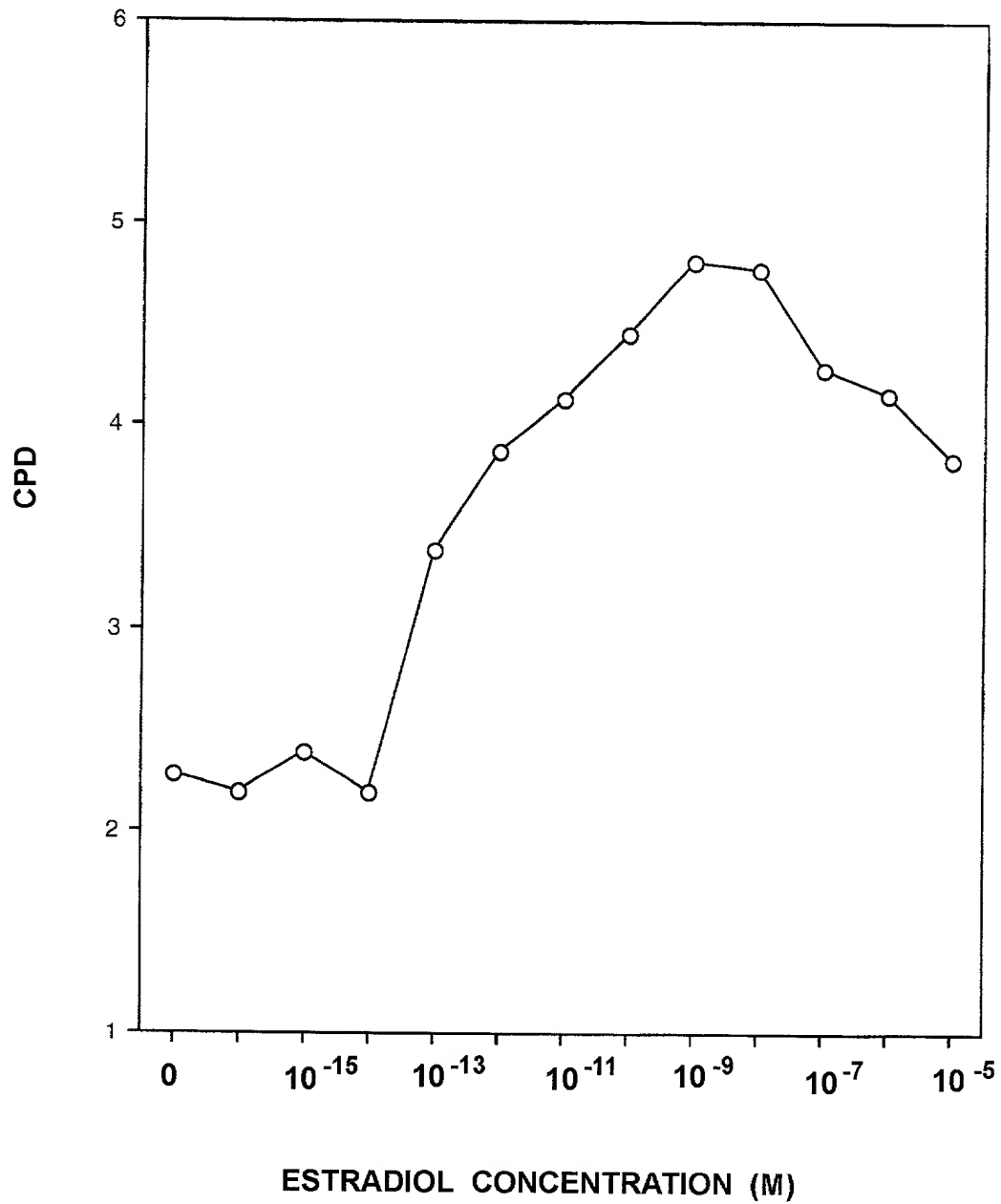
**EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON H301CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** Closed circles = + E<sub>2</sub>  
 Open circles = - E<sub>2</sub>  
 Closed triangles = Estrogenic effect

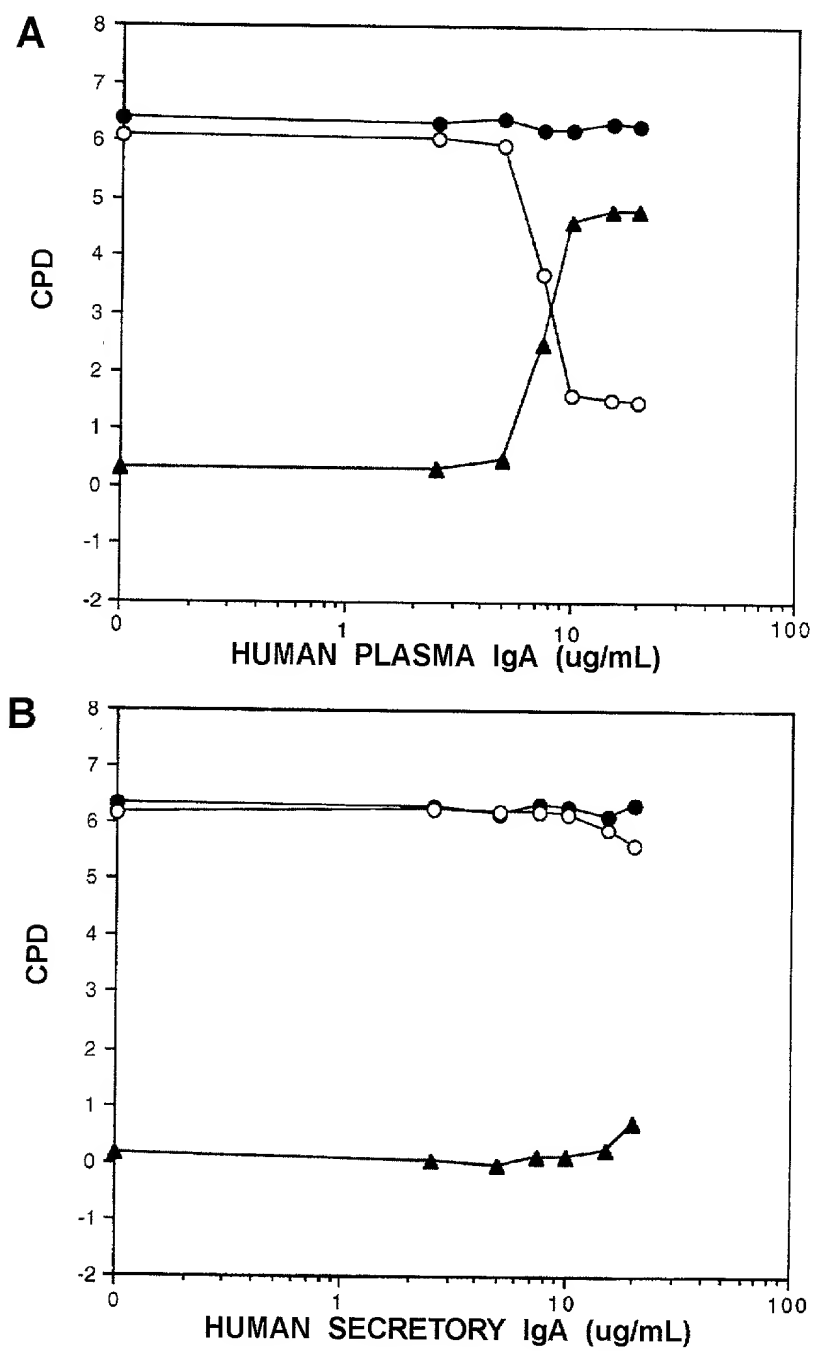
**FIGURE 92**

**EFFECT OF ESTRADIOL ON H301 CELL GROWTH IN  
SERUM-FREE MEDIUM AND 40 ug/mL OF HUMAN IgM**



**FIGURE 93**

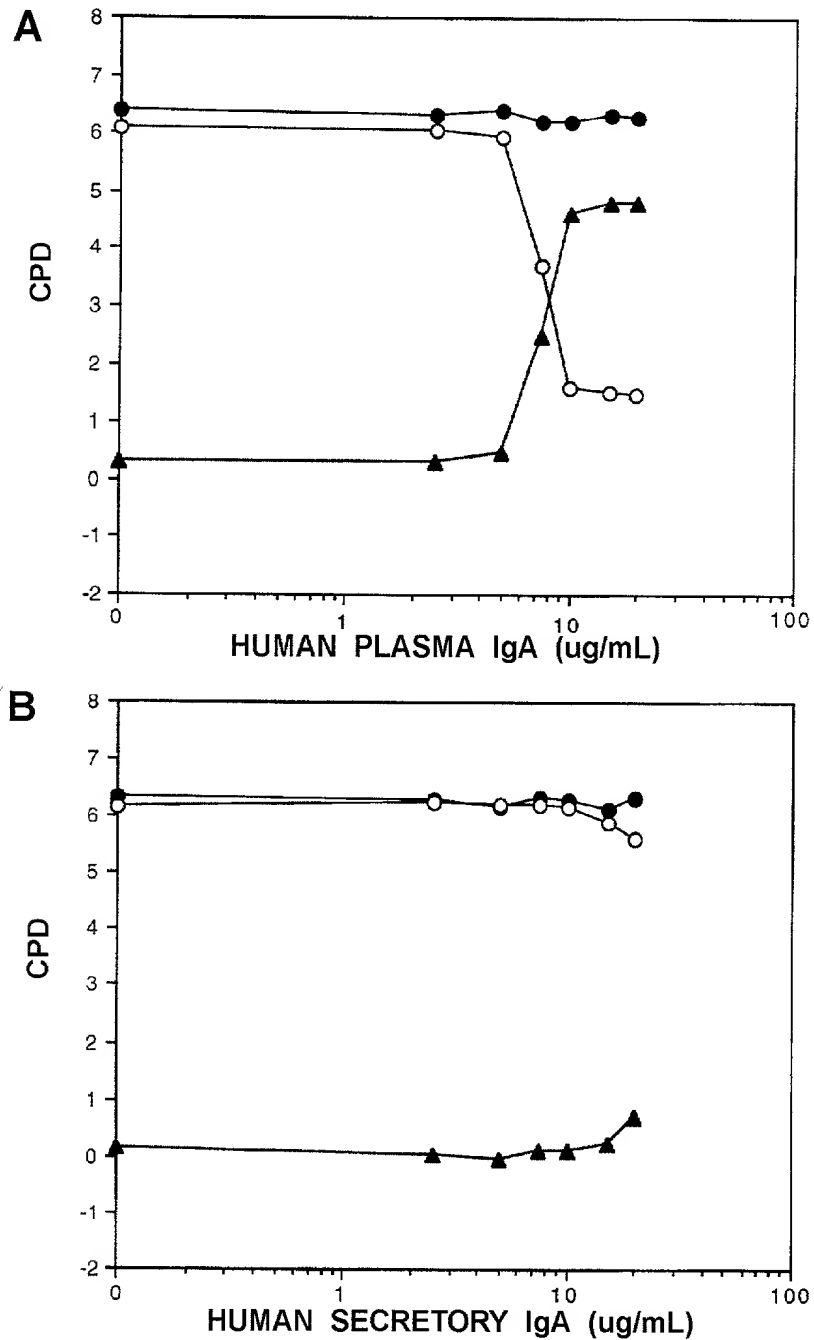
**EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF -7K CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** Closed circles = + E<sub>2</sub>  
Open circles = - E<sub>2</sub>  
Closed triangles = Estrogenic effect

**FIGURE 94**

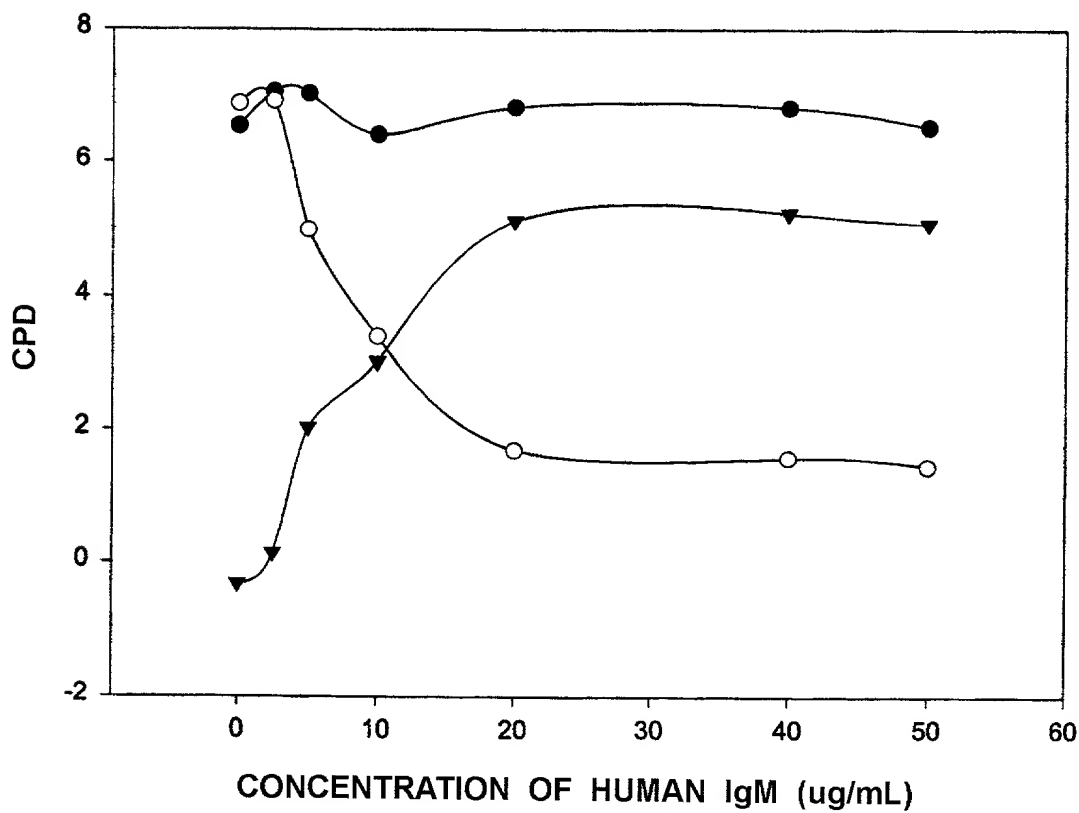
**EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** Closed circles = + E<sub>2</sub>  
 Open circles = - E<sub>2</sub>  
 Closed triangles = Estrogenic effect

**FIGURE 95**

**EFFECT OF HUMAN IgM ON MCF-7A CELL  
GROWTH IN SERUM-FREE MEDIUM**

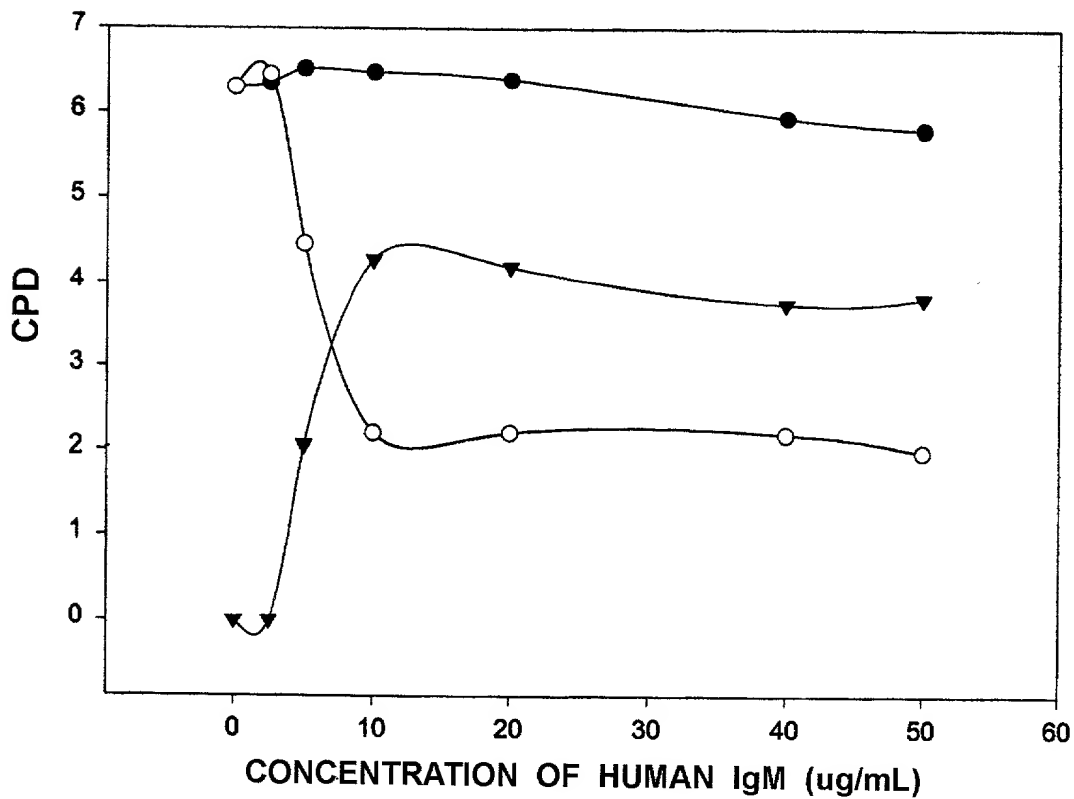


**LEGEND:**

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▼ = Estrogenic effect

FIGURE 96

EFFECT OF HUMAN IgM ON MCF-7K  
CELL GROWTH IN SERUM-FREE MEDIUM

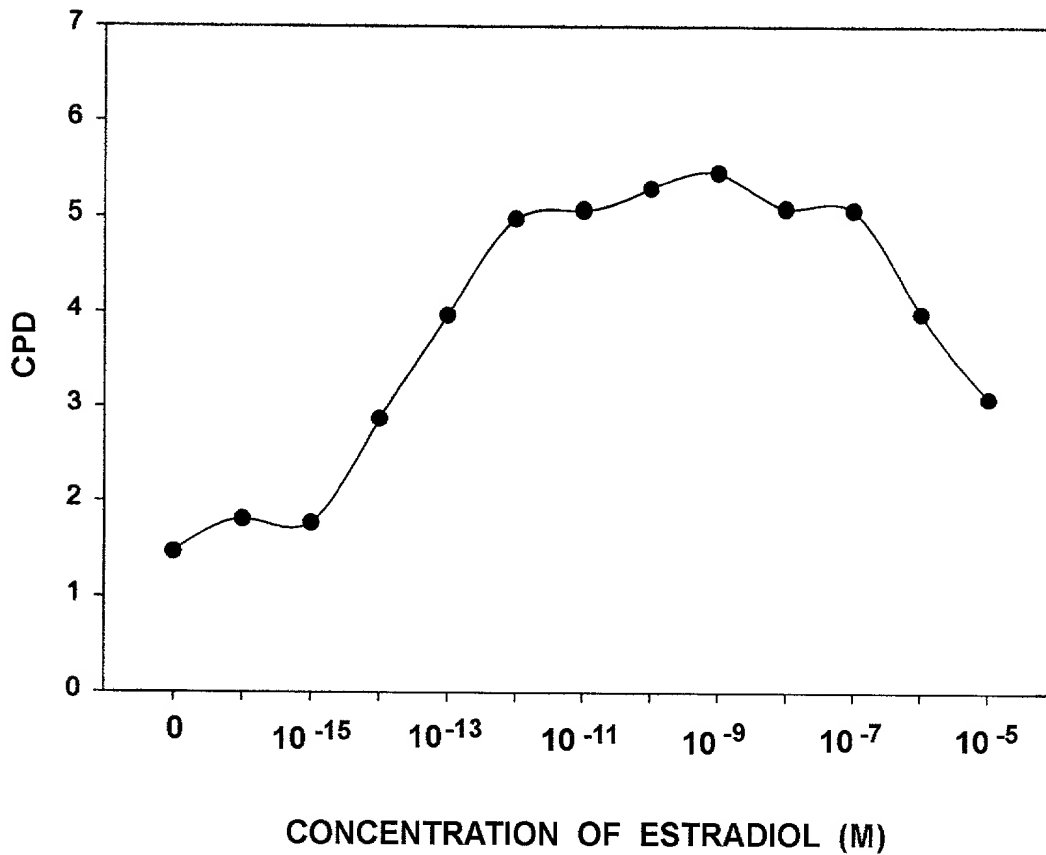


LEGEND:

- = + E<sub>2</sub>
- = - E<sub>2</sub>
- ▼ = Estrogenic effect

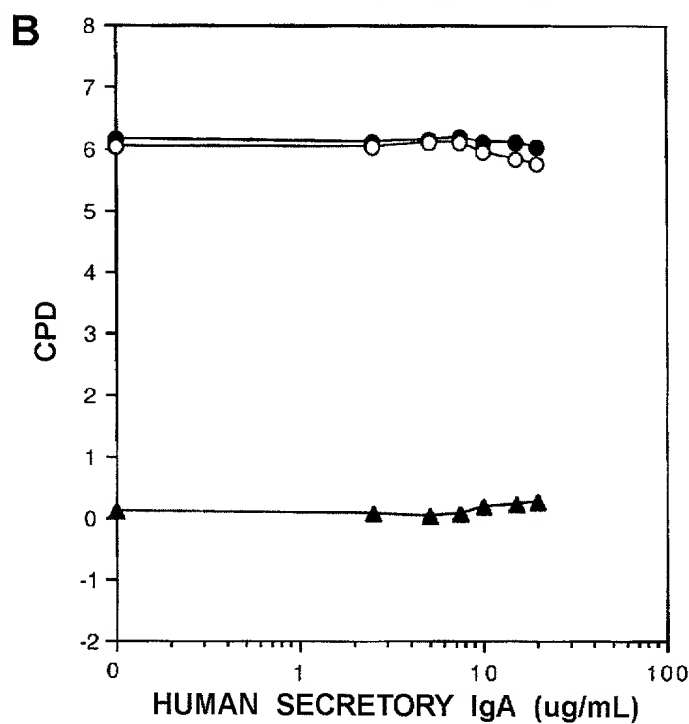
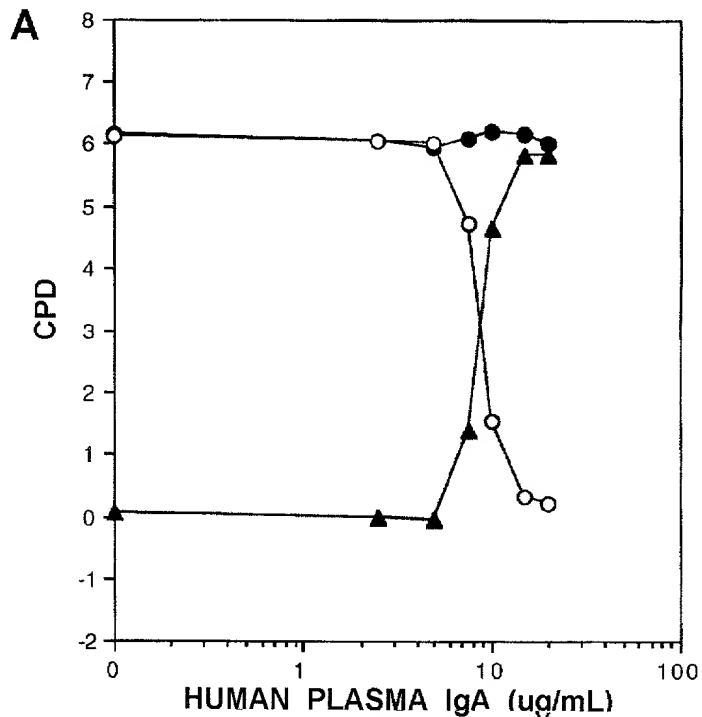
FIGURE 97

EFFECT OF ESTRADIOL ON MCF-7K CELL GROWTH  
IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM



**FIGURE 98**

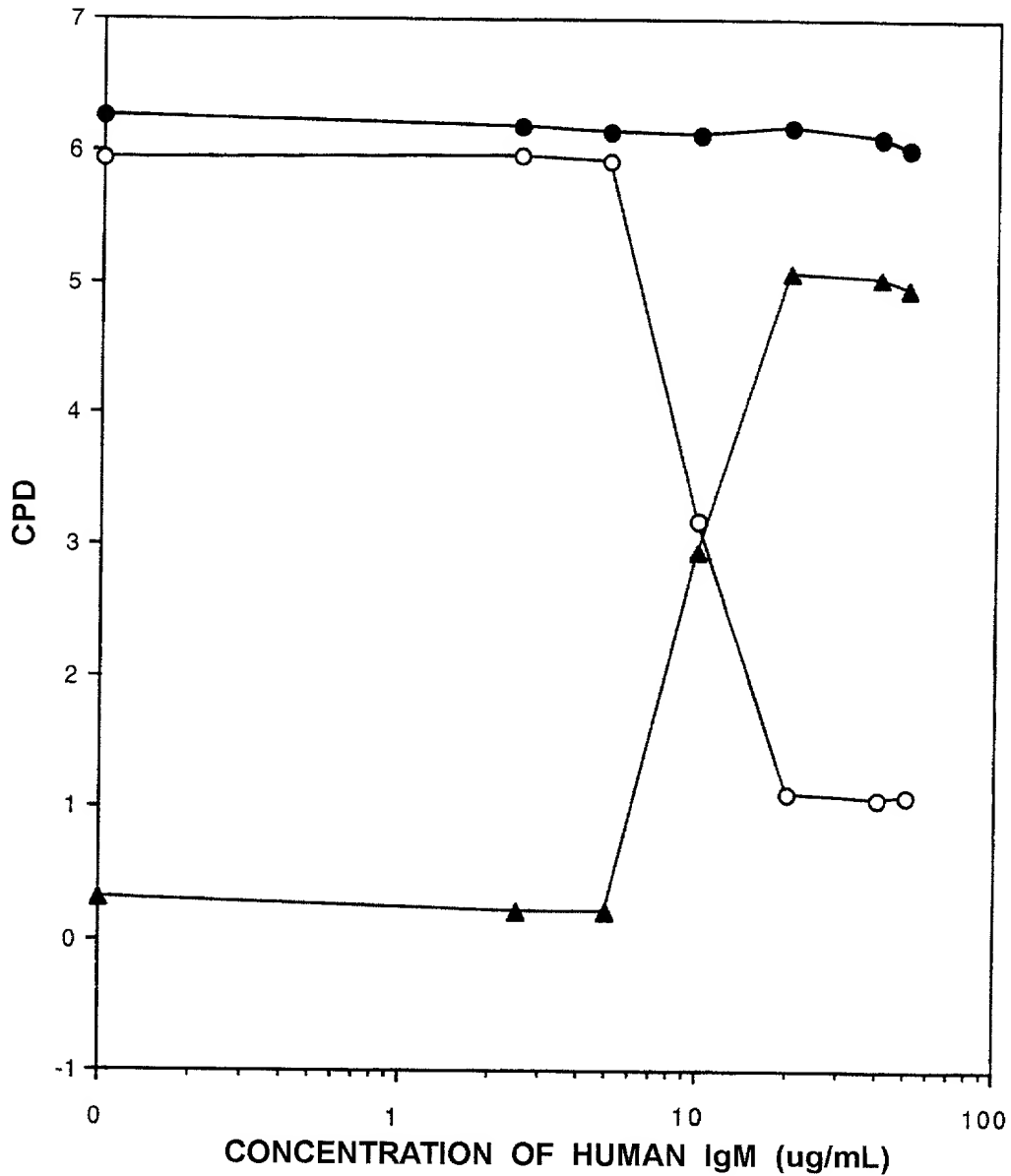
**EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON T47D CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** Closed circles = + E<sub>2</sub>  
 Open circles = - E<sub>2</sub>  
 Closed triangles = Estrogenic effect

**FIGURE 99**

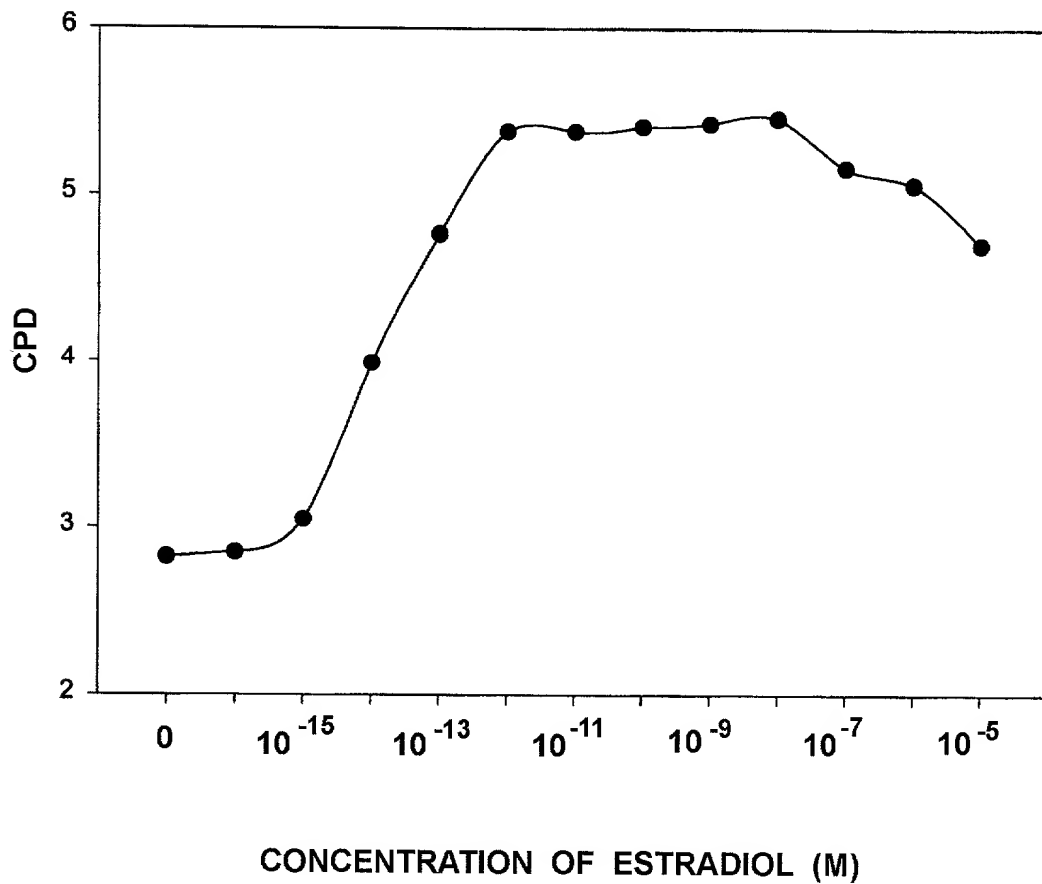
**EFFECT OF HUMAN IgM ON T47D CELL  
GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:**    Closed circles = + E<sub>2</sub>  
              Open circles = - E<sub>2</sub>  
              Closed triangles = Estrogenic effect

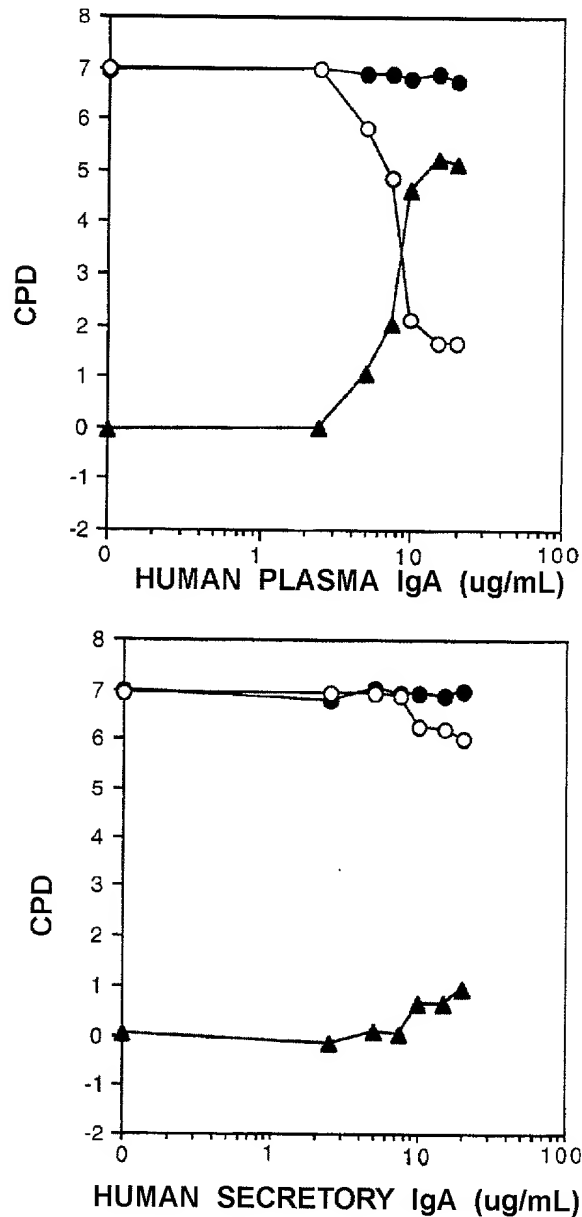
FIGURE 100

EFFECT OF ESTRADIOL ON T47D CELL GROWTH IN  
SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM



**FIGURE 101**

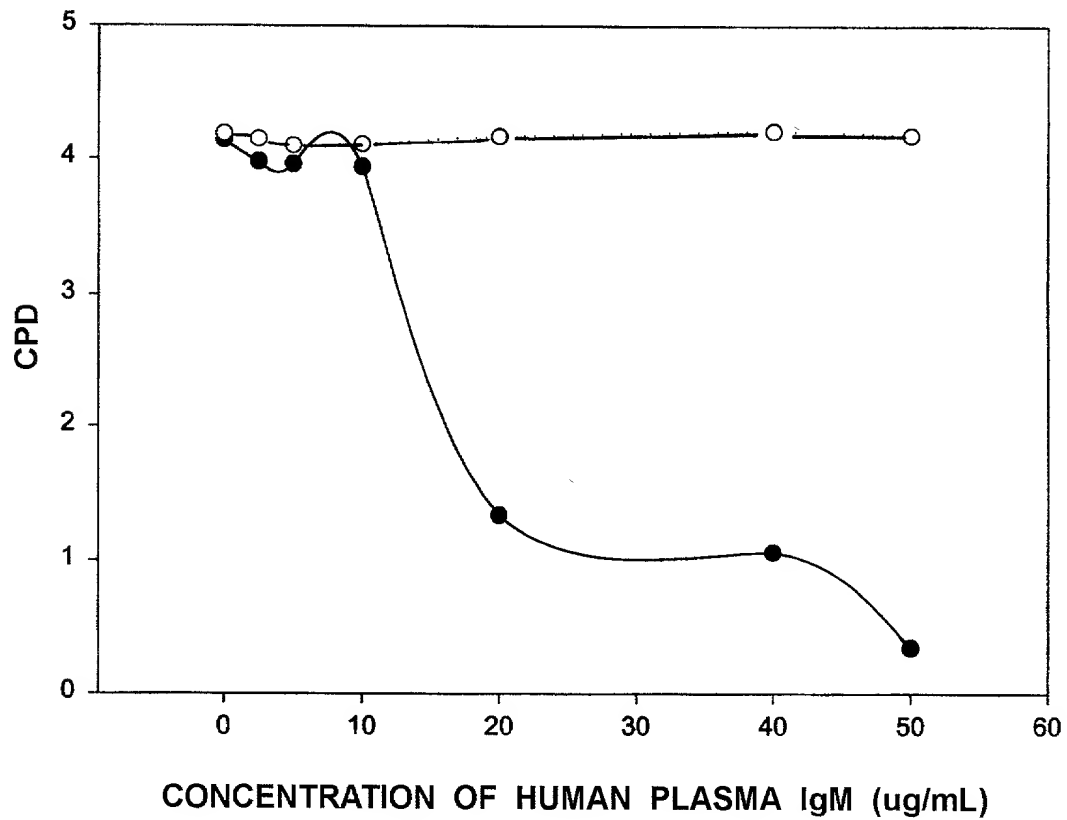
**EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** Closed circles = + E<sub>2</sub>  
 Open circles = - E<sub>2</sub>  
 Closed triangles = Estrogenic effect

**FIGURE 102**

**EFFECT OF HUMAN PLASMA IgM ON  
 ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM**

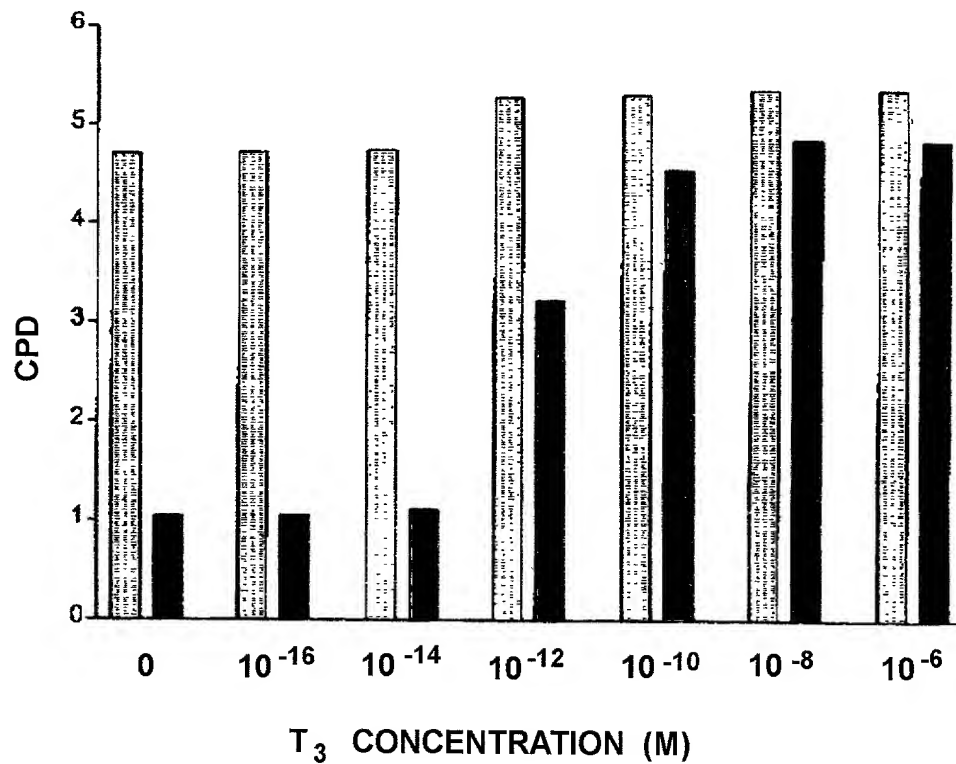


**LEGEND:**

—●— = - E<sub>2</sub>  
 —○— = + E<sub>2</sub>

FIGURE 103

EFFECT OF HUMAN IgM ON HT-29 CELL GROWTH IN  
 THE PRESENCE OF INCREASING CONCENTRATIONS OF  $T_3$



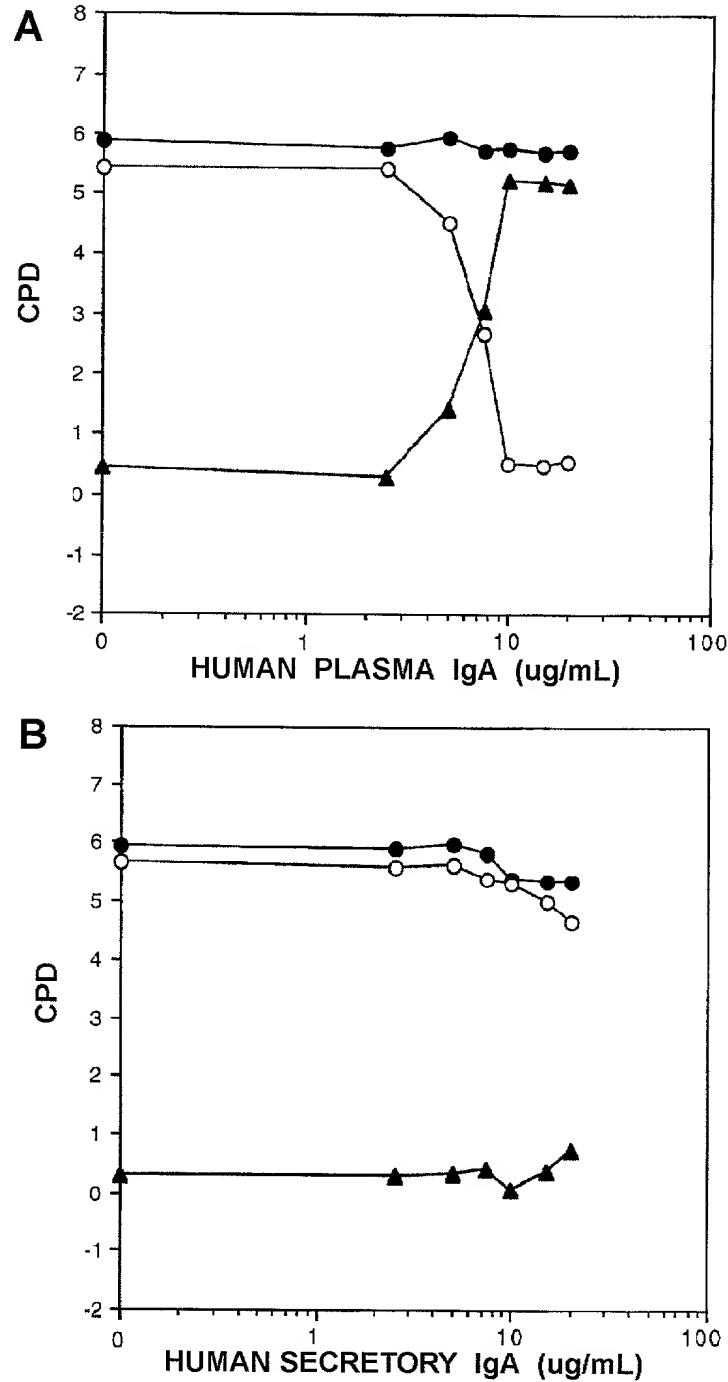
LEGEND:

□ =  $T_3$  Titration

■ =  $T_3$  Titration + 40 ug/mL IgM

**FIGURE 104**

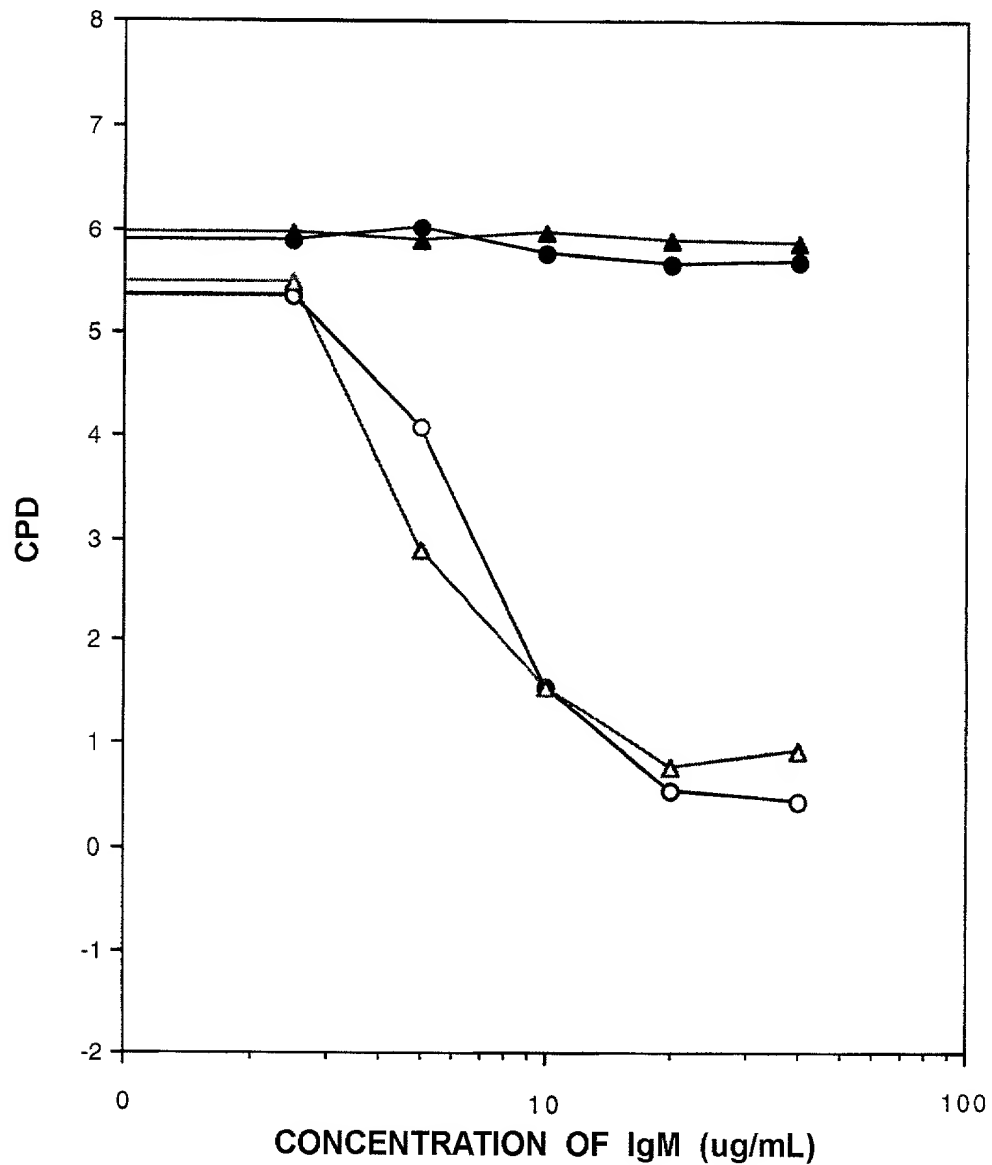
**EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** Closed circles = + E<sub>2</sub>  
 Open circles = - E<sub>2</sub>  
 Closed triangles = Estrogenic effect

**FIGURE 105**

**EFFECTS OF HUMAN PLASMA IgM VS IgM DERIVED FROM MYELOMA CELLS ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM WITH AND WITHOUT DHT**

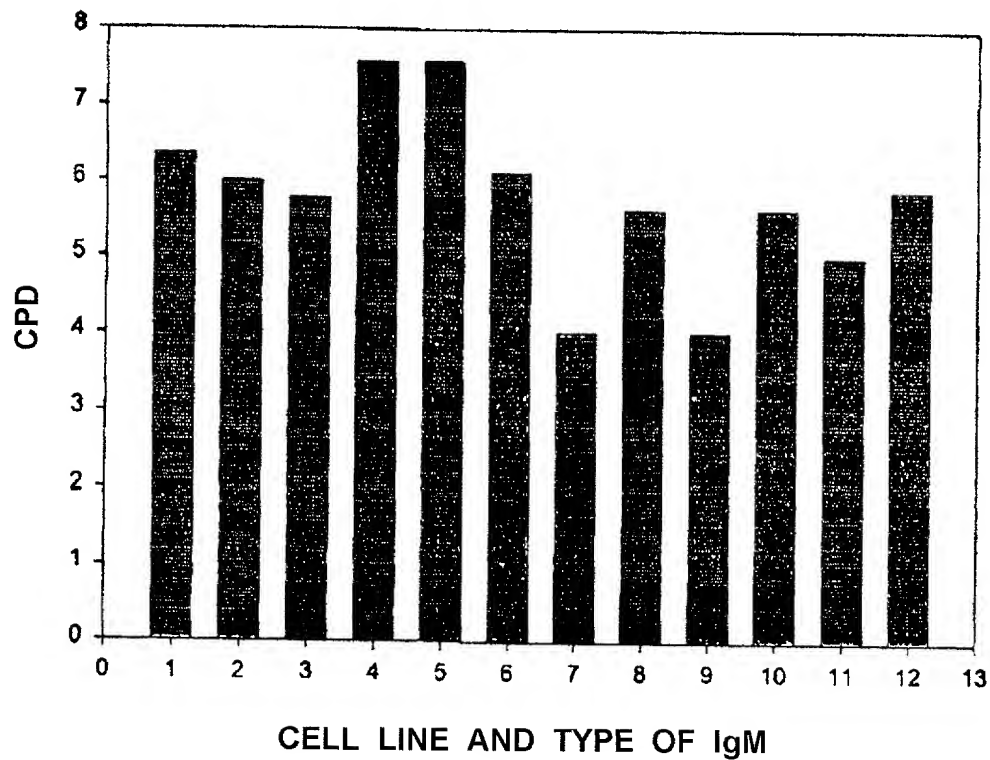


**LEGEND:**

- = DHT + Myeloma IgM
- = Myeloma IgM only
- ▲— = DHT + Plasma IgM
- △— = Plasma IgM only

**FIGURE 106**

**ESTROGENIC EFFECT OF 50 ug/mL OF VARIOUS  
IgM'S ON SEVERAL DIFFERENT CELL LINES**

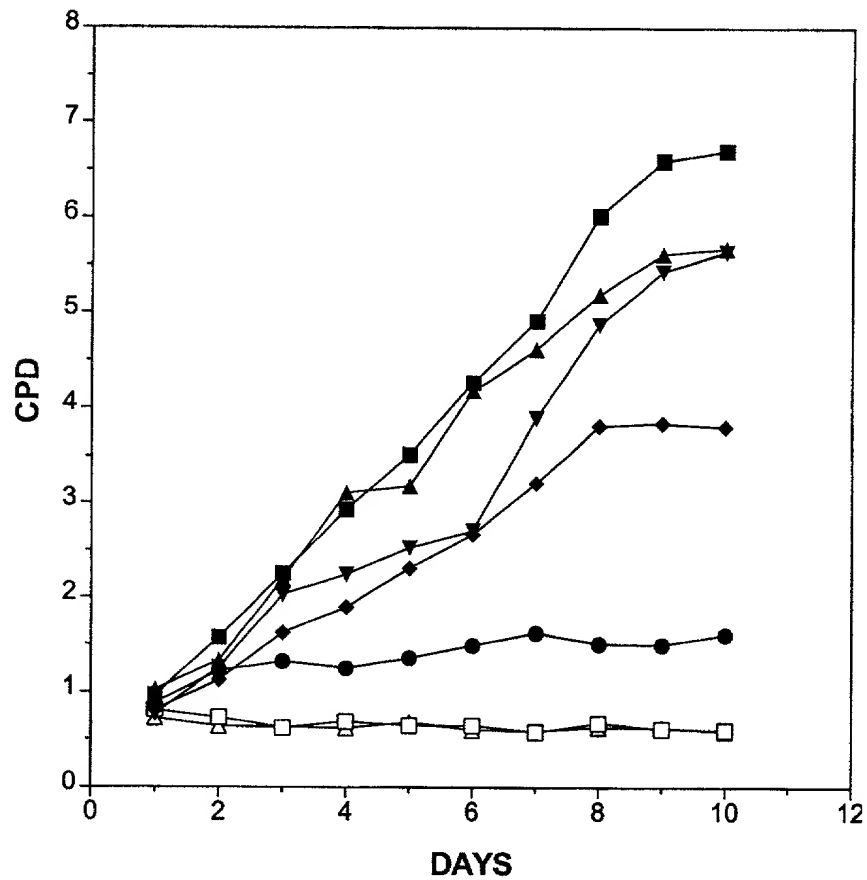


**LEGEND:**

1. Human IgM on MTW9/PL2 Cells = 6.36 cpd
2. Mouse IgM on MTW9/PL2 Cells = 6.00 cpd
3. Rat IgM on MTW9/PL2 Cells = 5.77 cpd
4. Human IgM on H301 Cells = 7.57 cpd
5. Mouse IgM on H301 Cells = 7.56 cpd
6. Rat IgM on H301 Cells = 6.11 cpd
7. Human IgM on GH1 Cells = 4.12 cpd
8. Rat IgM on GH1 Cells = 5.83 cpd
9. Human IgM on GH3 Cells = 4.09 cpd
10. Human IgM on GH4 Cells = 5.41 cpd
11. Human IgM on MCF-7A Cells = 5.01 cpd
12. Human IgM on MCF-7K Cells = 5.89 cpd

**FIGURE 107**

**EFFECT OF TAMOXIFEN ON T47D CELL GROWTH  
 IN DDM-2MF DEFINED MEDIUM**

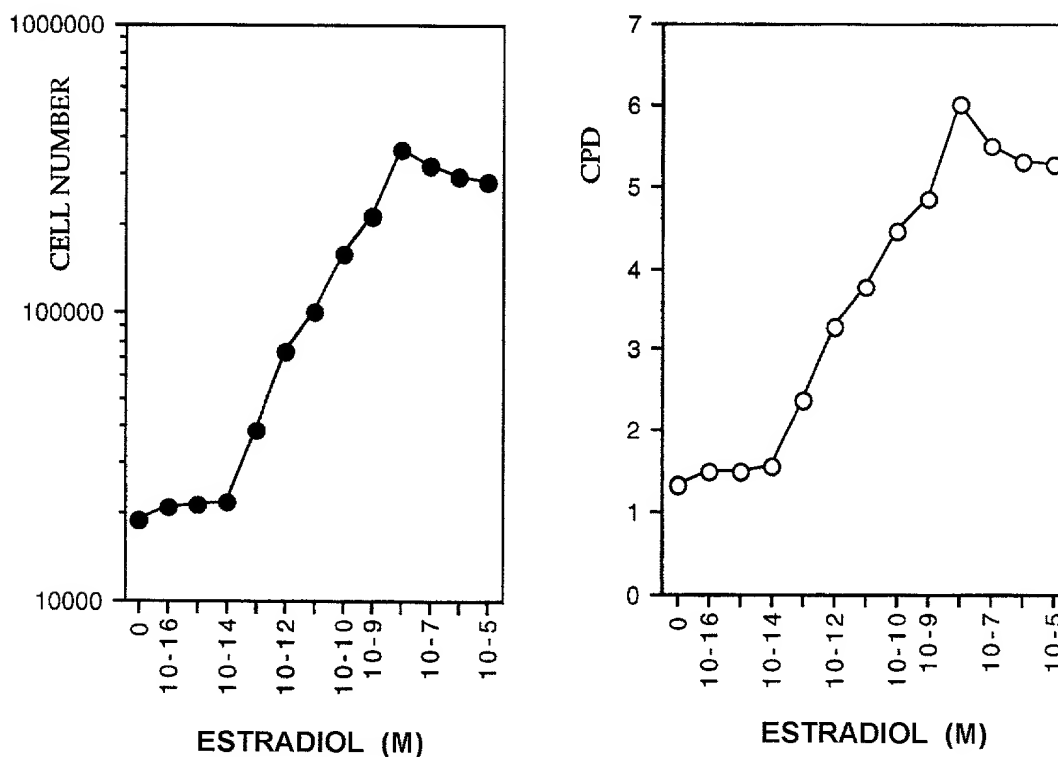


**LEGEND:**

- SFM + E<sub>2</sub>
- ▲ SFM - E<sub>2</sub>
- ▼ SFM + 10<sup>-9</sup> M TAM
- ◆ SFM + 10<sup>-8</sup> M TAM
- SFM + 10<sup>-7</sup> M TAM
- SFM + 10<sup>-6</sup> M TAM
- △ SFM + 10<sup>-5</sup> M TAM

**FIGURE 108**

**EFFECT OF INCREASING ESTRADIOL CONCENTRATIONS  
 ON T47D CELL GROWTH IN SERUM-FREE AND  
 PHENOL-RED FREE MEDIUM WITH  $10^{-7}$  TAMOXIFEN**

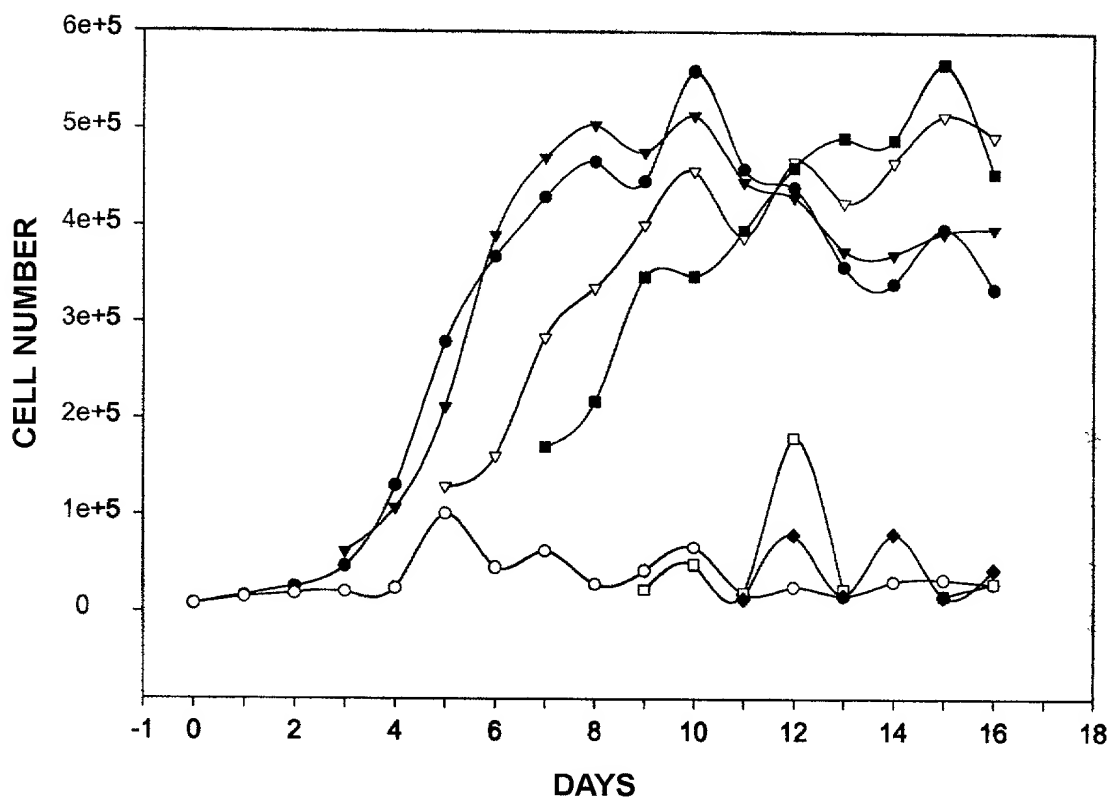


**NOTE:**

**DATA ARE EXPRESSED AS BOTH CELL NUMBER AND CPD**

FIGURE 109

**E<sub>2</sub> RESCUE OF MTW9/PL2 CELL GROWTH IN  
SERUM-FREE MEDIUM WITH 40 ug/mL HORSE IgM**



LEGEND:

- = E<sub>2</sub> Added on Day 0
- = No E<sub>2</sub>
- ▼— = E<sub>2</sub> Added on Day 2
- ▽— = E<sub>2</sub> Added on Day 4
- = E<sub>2</sub> Added on Day 6
- = E<sub>2</sub> Added on Day 8
- ◆— = E<sub>2</sub> Added on Day 10

**FIGURE 110**

**SUMMARY OF E<sub>2</sub> RESCUE OF MTW9/PL2 CELL GROWTH  
IN SERUM-FREE MEDIUM WITH 40 ug/mL HORSE IgM**

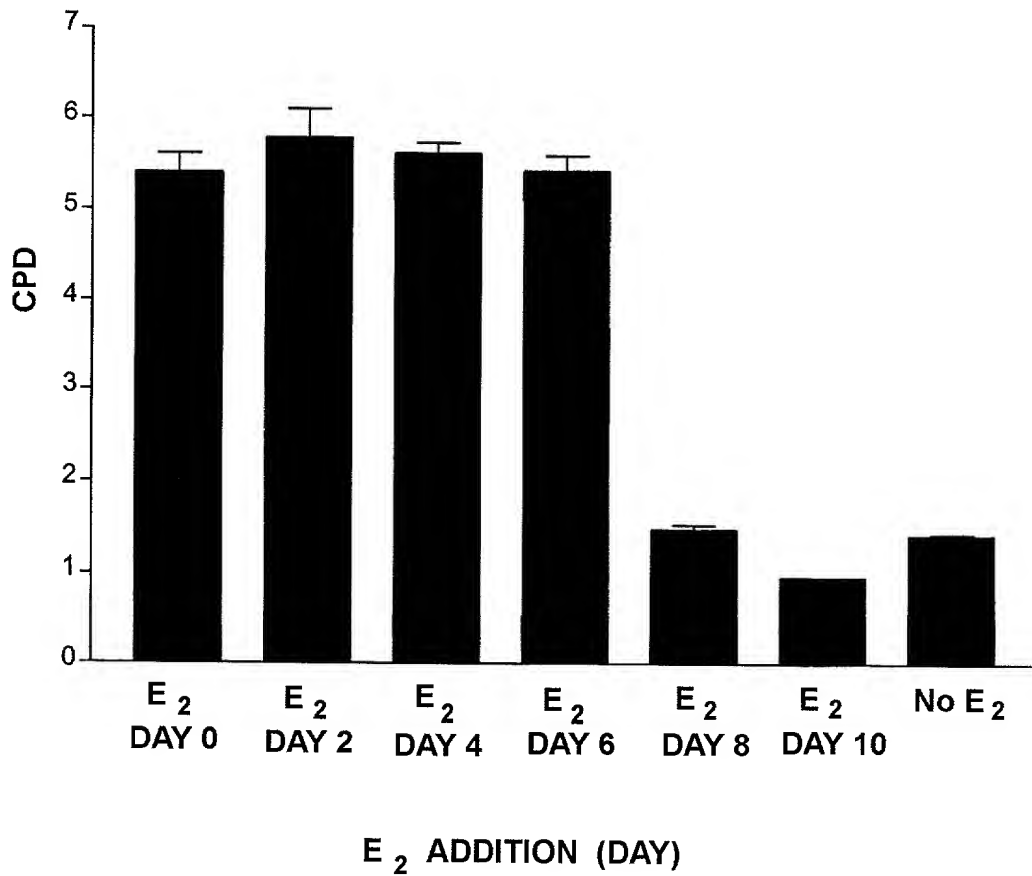
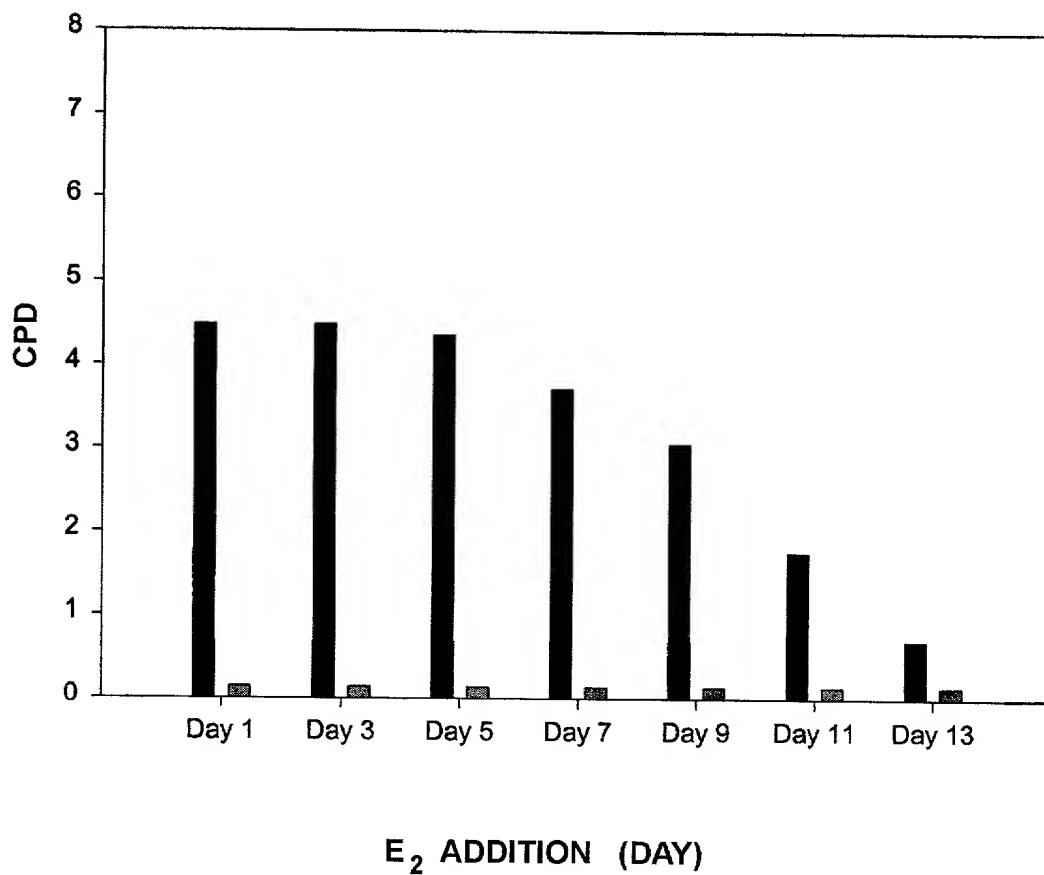


FIGURE 111

**E<sub>2</sub> RESCUE OF T47D CELL GROWTH IN SERUM-FREE  
MEDIUM WITH 40 ug/mL HORSE IgM**

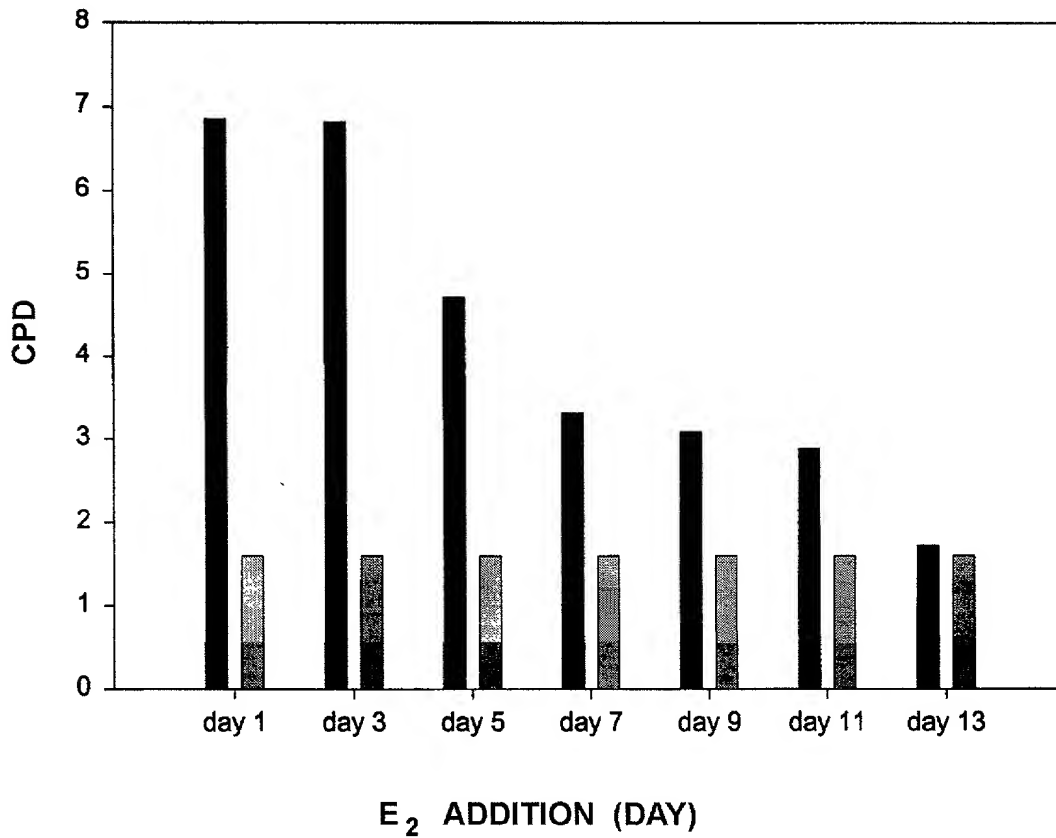


LEGEND:

■ = + E<sub>2</sub>  
▨ = - E<sub>2</sub>

**FIGURE 112**

**ESTROGEN RESCUE OF MCF-7A CELL GROWTH IN  
SERUM-FREE MEDIUM WITH 40 ug/mL OF HUMAN SERUM IgM**

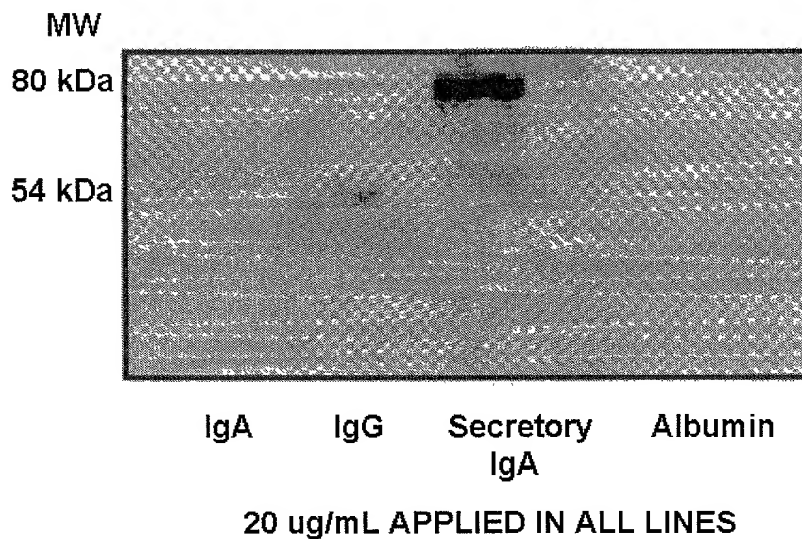


**LEGEND:**

■ = + E<sub>2</sub>  
▨ = - E<sub>2</sub>

## FIGURE 113

### DETECTION OF SECRETORY COMPONENT IN SECRETORY IgA WITH ANTI-SC ANTIBODY



IgA = Human Plasma

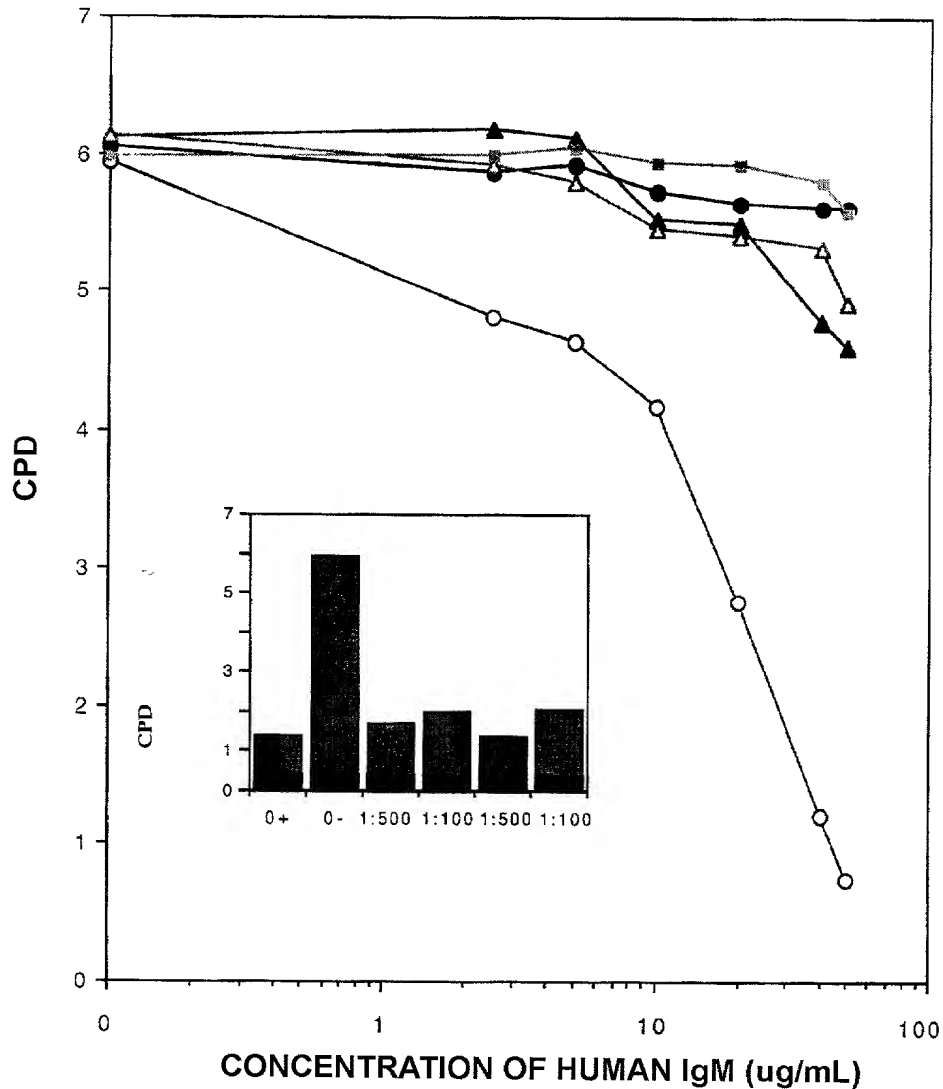
IgG = Human Plasma

Secretory IgA = IgA from Milk

Albumin = Human

**FIGURE 114**

**HUMAN IgM TITRATION ON T47D CELLS GROWN IN SERUM-FREE MEDIUM WITH DIFFERENT DILUTIONS OF ANTI-SC ANTIBODY**

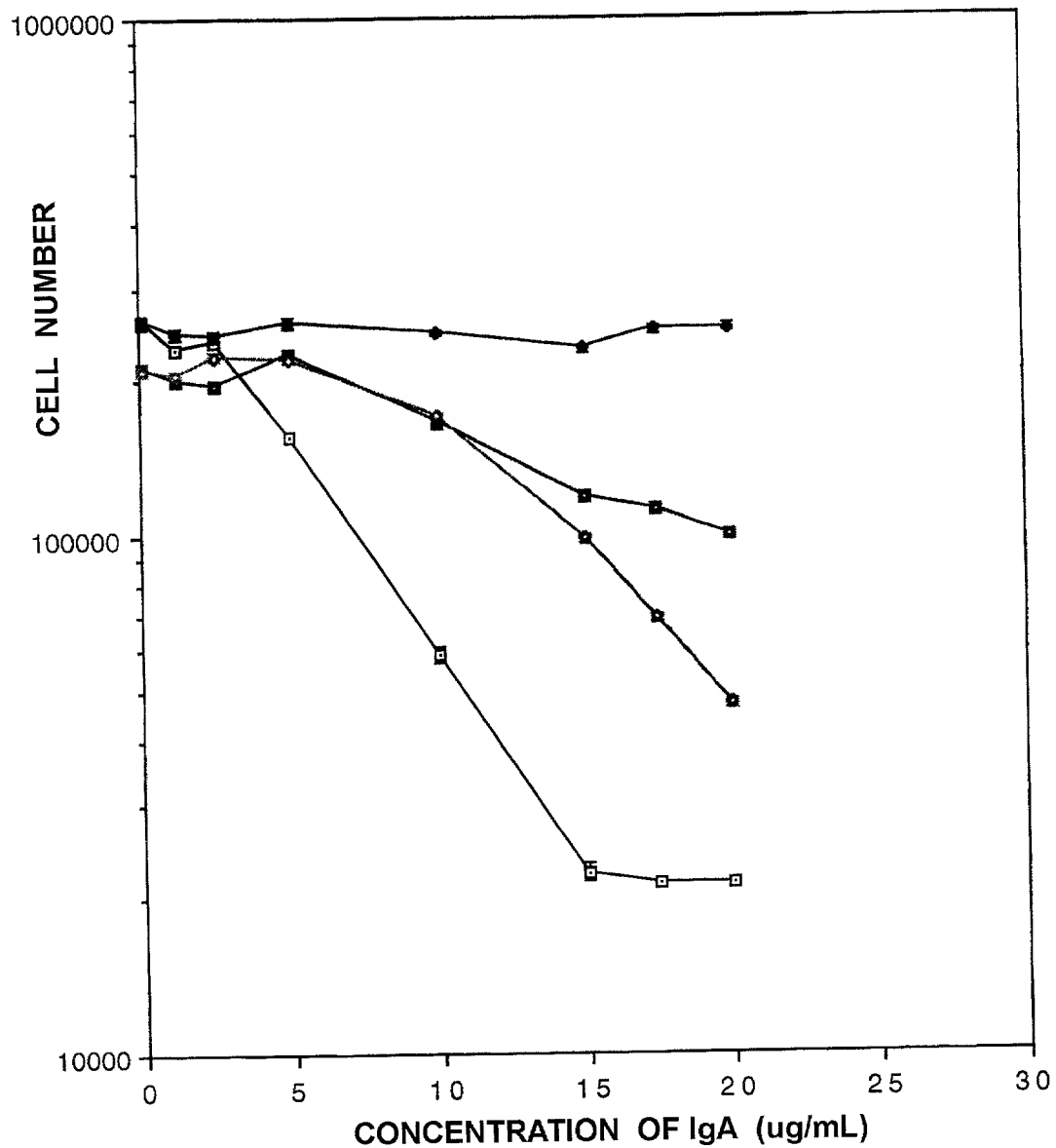


**LEGEND:** ● = + E<sub>2</sub>  
 ○ = - E<sub>2</sub>  
 ▲ = 1:5000 Dilution of Anti-SC Antibody  
 △ = 1:1000 Dilution of Anti-SC Antibody  
 ■ = 1:500 Dilution of Anti-SC Antibody

**INSERT: EFFECT OF RABBIT SERUM ON T47D CELLS INCUBATED WITH 40 ug/mL HUMAN IgM**

FIGURE 115

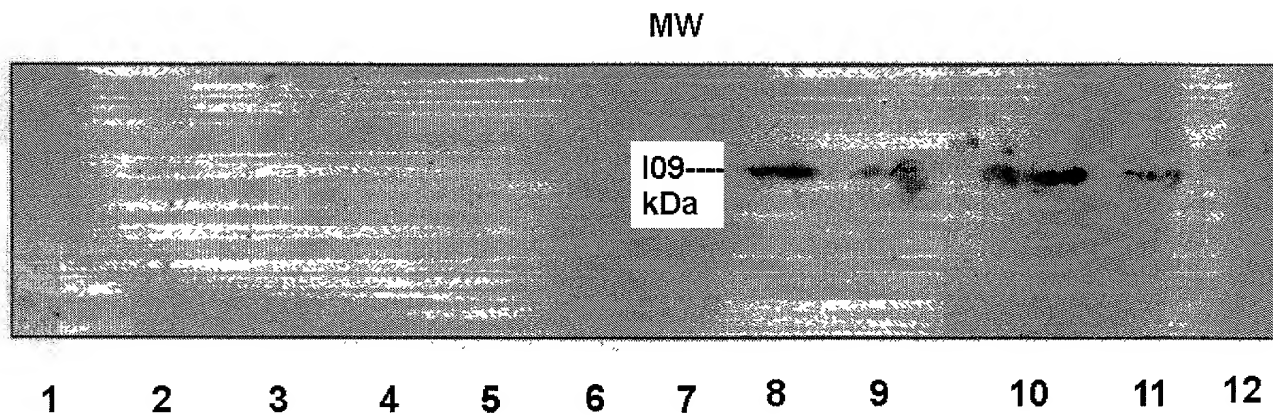
EFFECT OF IgA ON LNCaP GROWTH IN THE  
PRESENCE OF ANTI-SECRETORY COMPONENT  
ANTIBODY AT DIFFERENT DILUTIONS



LEGEND: —□— = Control  
—◆— = 1:100 Dilution of Anti-SC Antibody  
—■— = 1:500 Dilution of Anti-SC Antibody  
—○— = 1:1000 Dilution of Anti-SC Antibody

**FIGURE 116**

**WESTERN BLOT: ANTI-SECRETORY COMPONENT**

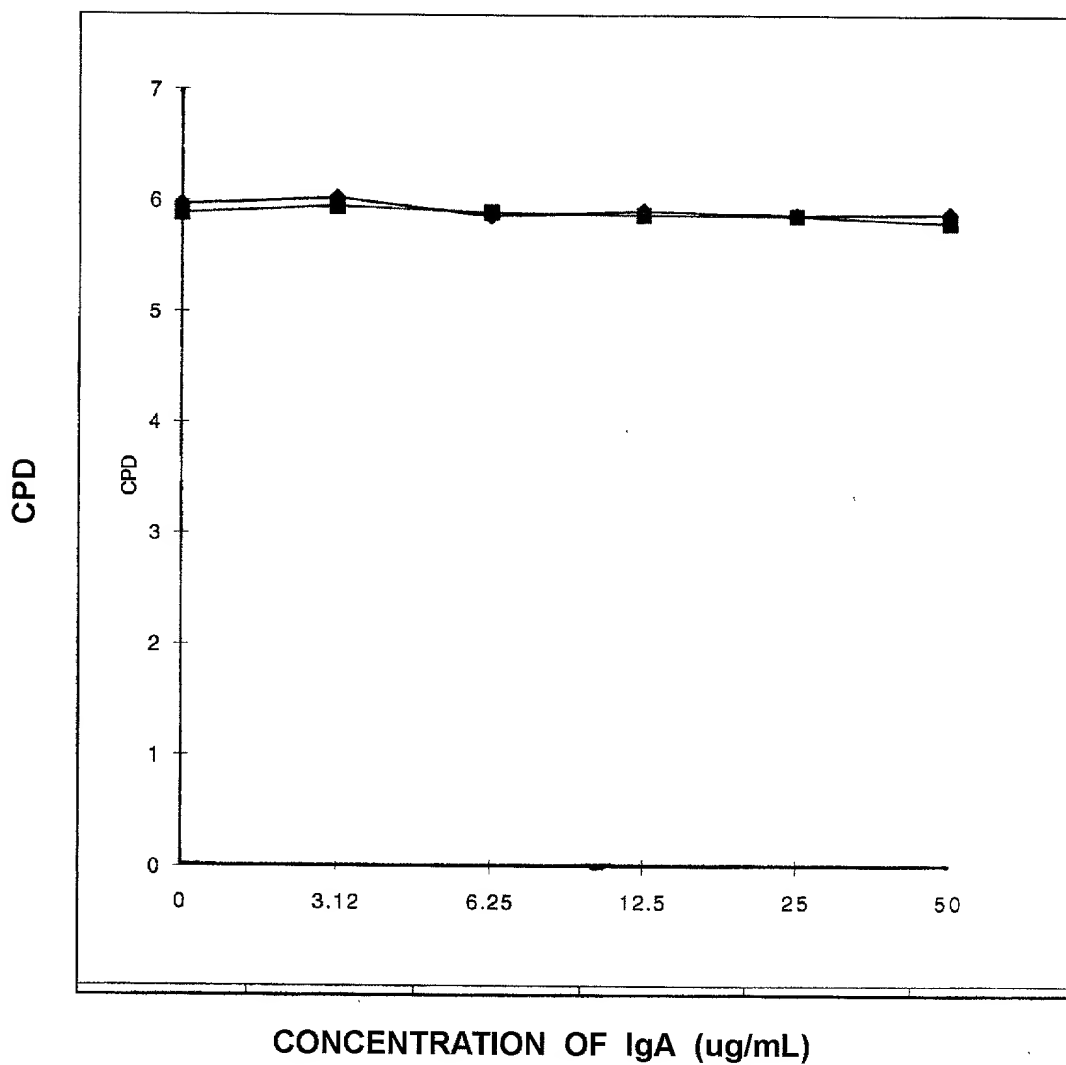


**LEGEND:**

1. MW
2. ALVA 41: 40 ug
3. ALVA 41: 20 ug
4. DU 145: 40 ug
5. DU 145: 20 ug
6. HUMAN FIBROBLAST: 40 ug
7. HUMAN FIBROBLAST: 20 ug
8. LNCaP: 40 ug
9. LNCaP: 20 ug
10. MDCK1: 20 ug
11. MDCK1: 10 ug
12. PC3: 40 ug

FIGURE 117

EFFECT OF HUMAN PLASMA IgA ON DU145  
CELL GROWTH WITH AND WITHOUT DHT



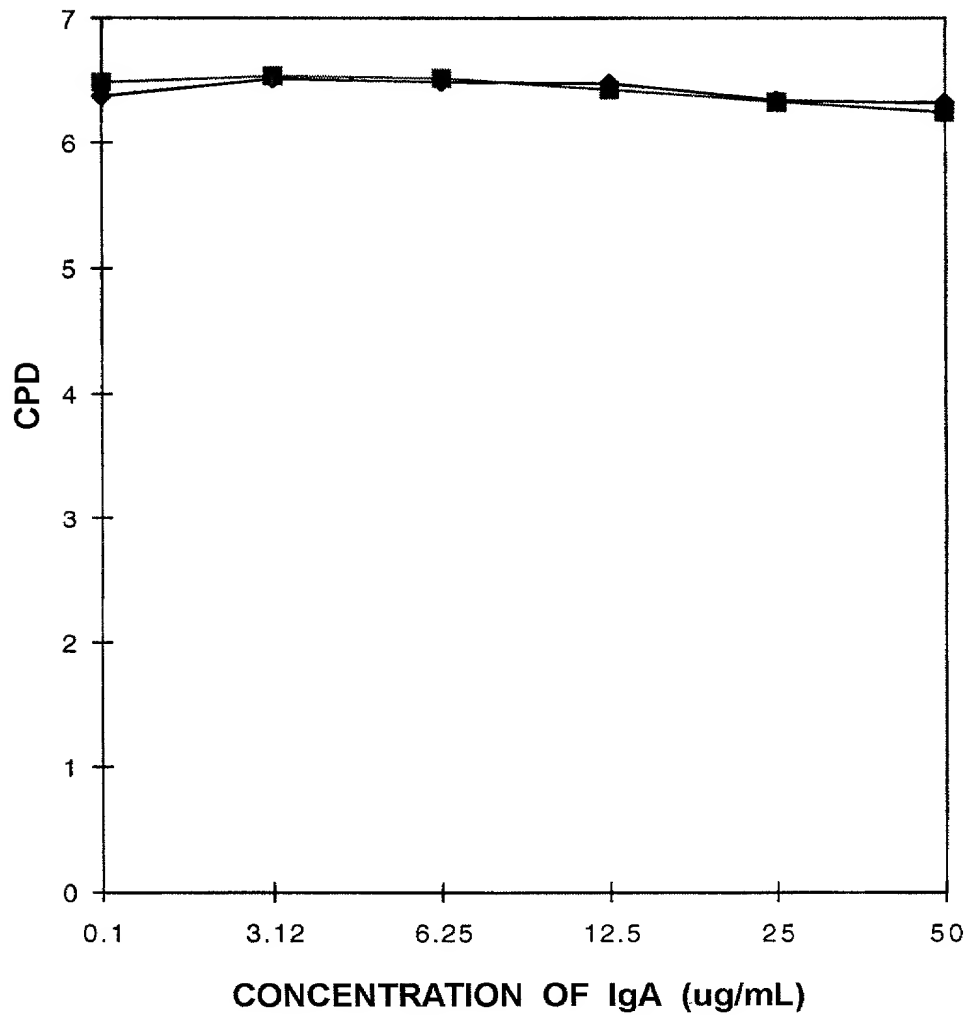
LEGEND:

—◆— = + DHT

—■— = - DHT

**FIGURE 118**

**EFFECT OF HUMAN PLASMA IgA ON PC3  
CELL GROWTH WITH AND WITHOUT DHT**



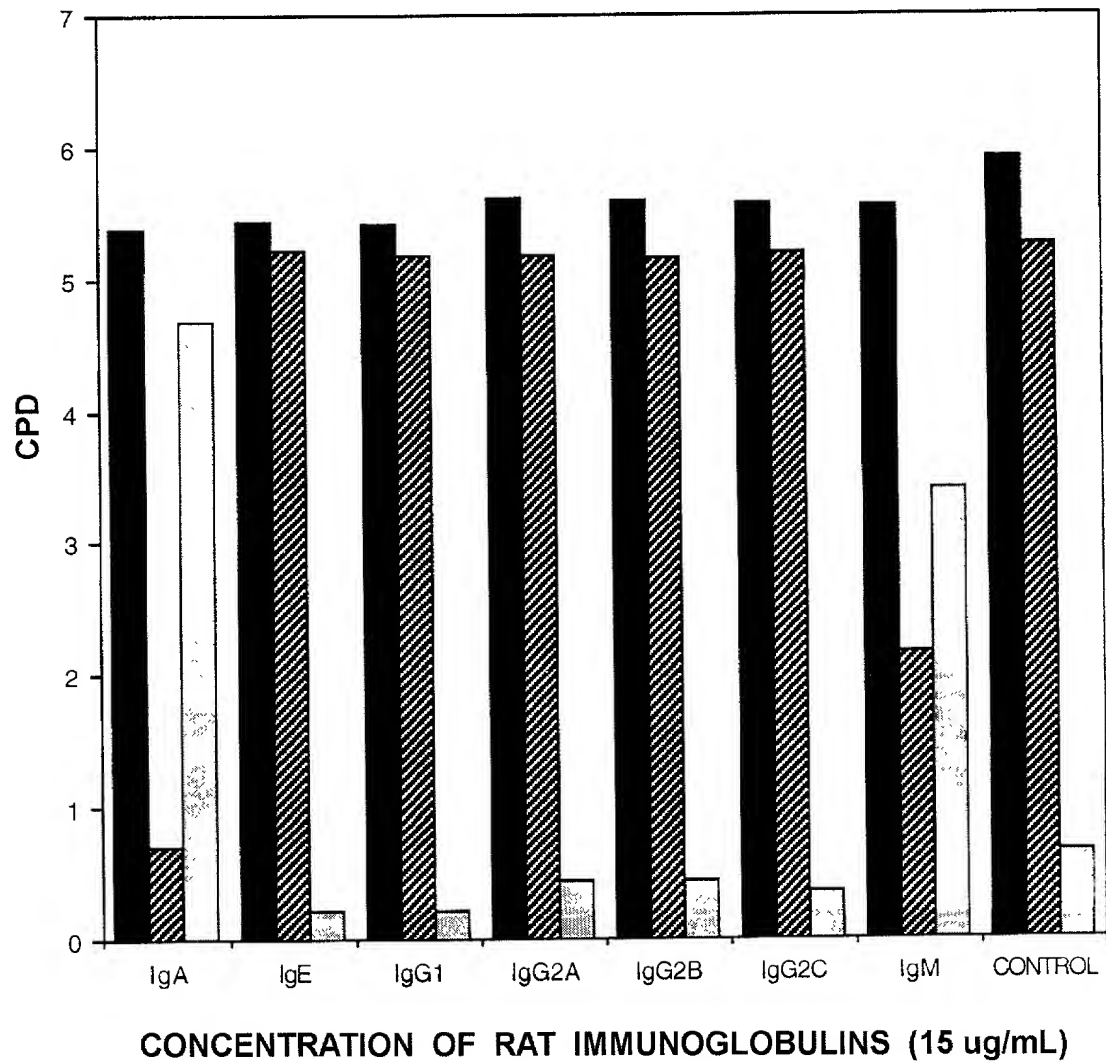
**LEGEND:**

—◆— = + DHT

—■— = - DHT

**FIGURE 119**

**EFFECT OF RAT IMMUNOGLOBULINS ON MTW9/PL2  
 CELL GROWTH IN SERUM-FREE MEDIUM**



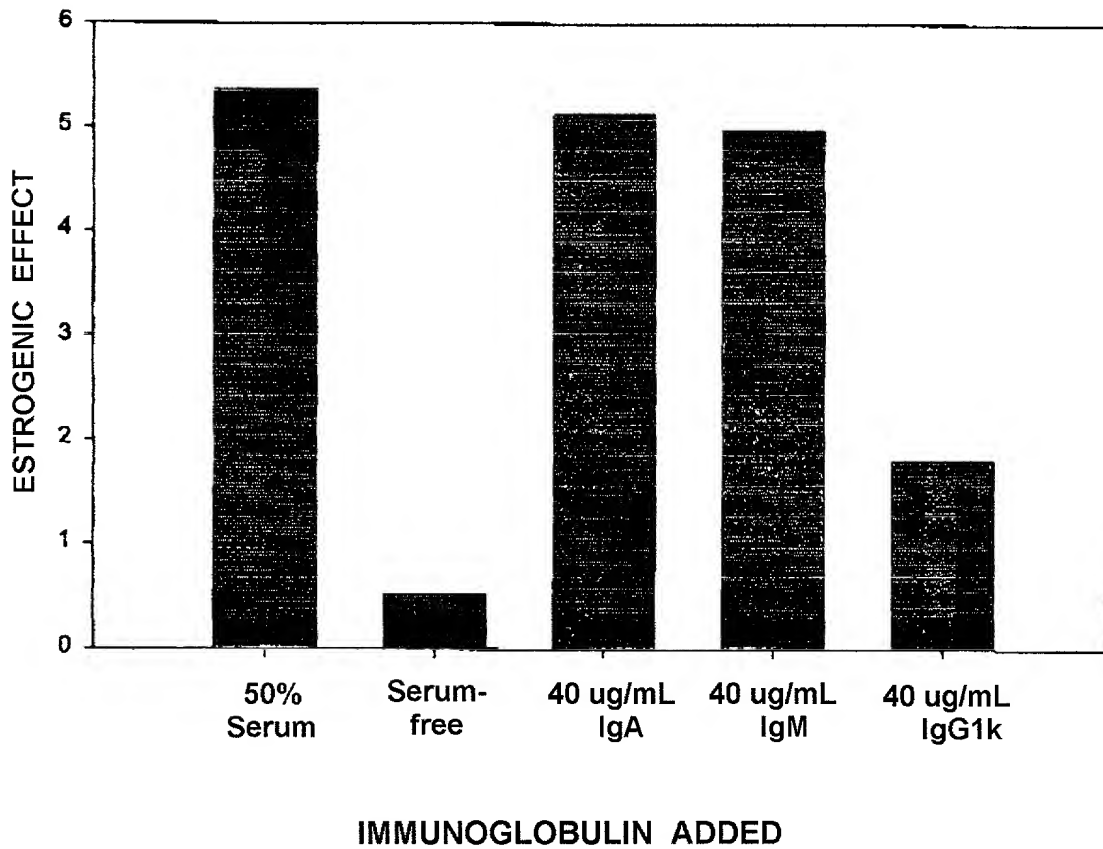
**LEGEND:**

- = + E<sub>2</sub>
- ▨ = - E<sub>2</sub>
- = Estrogenic effect

CONTROL IS SERUM-FREE MEDIUM ALONE  $\pm$  E<sub>2</sub>

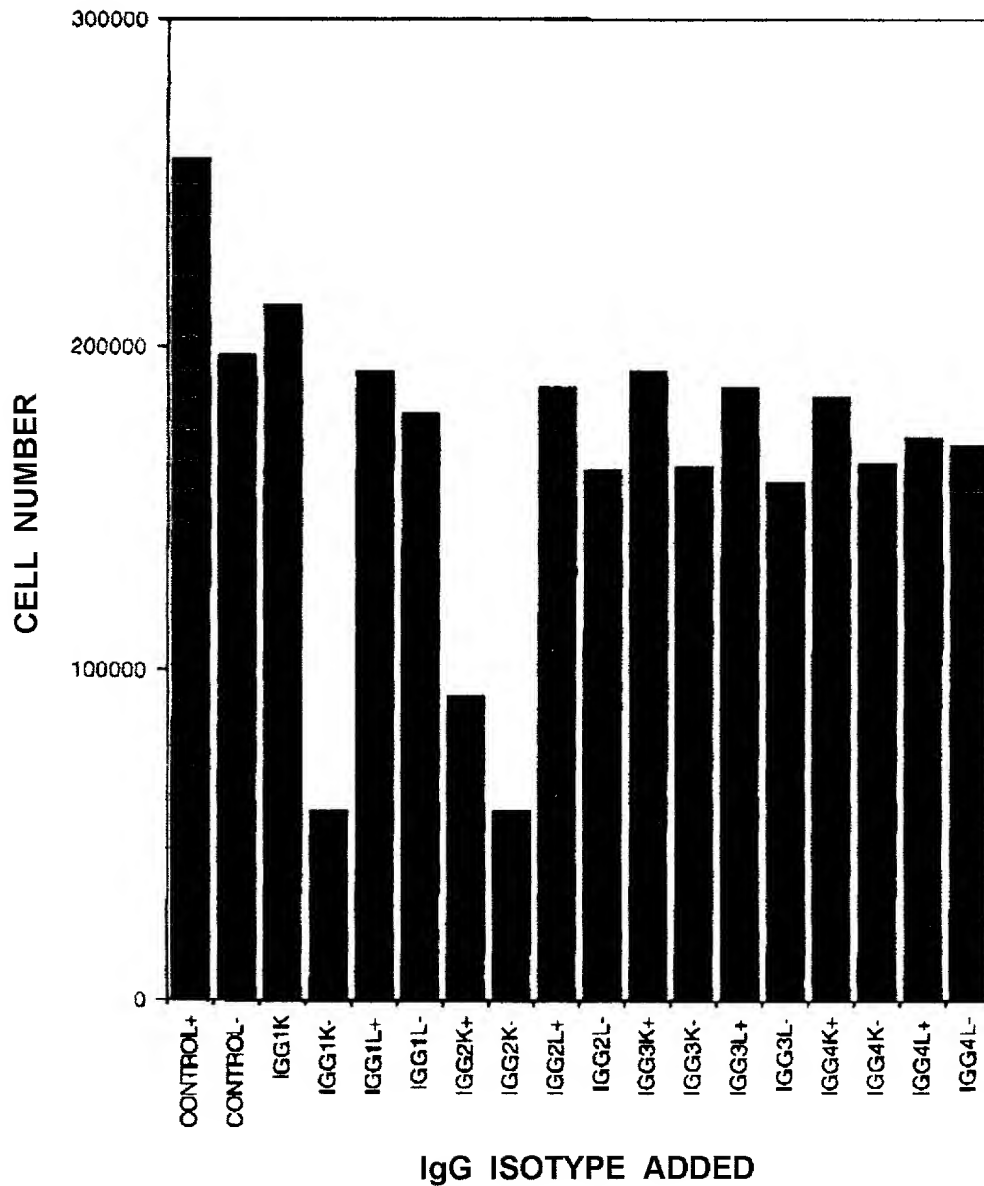
**FIGURE 120**

**ESTROGENIC EFFECT GENERATED BY IMMUNOGLOBULINS  
WITH T47D CELLS IN SERUM-FREE MEDIUM**



**FIGURE 121**

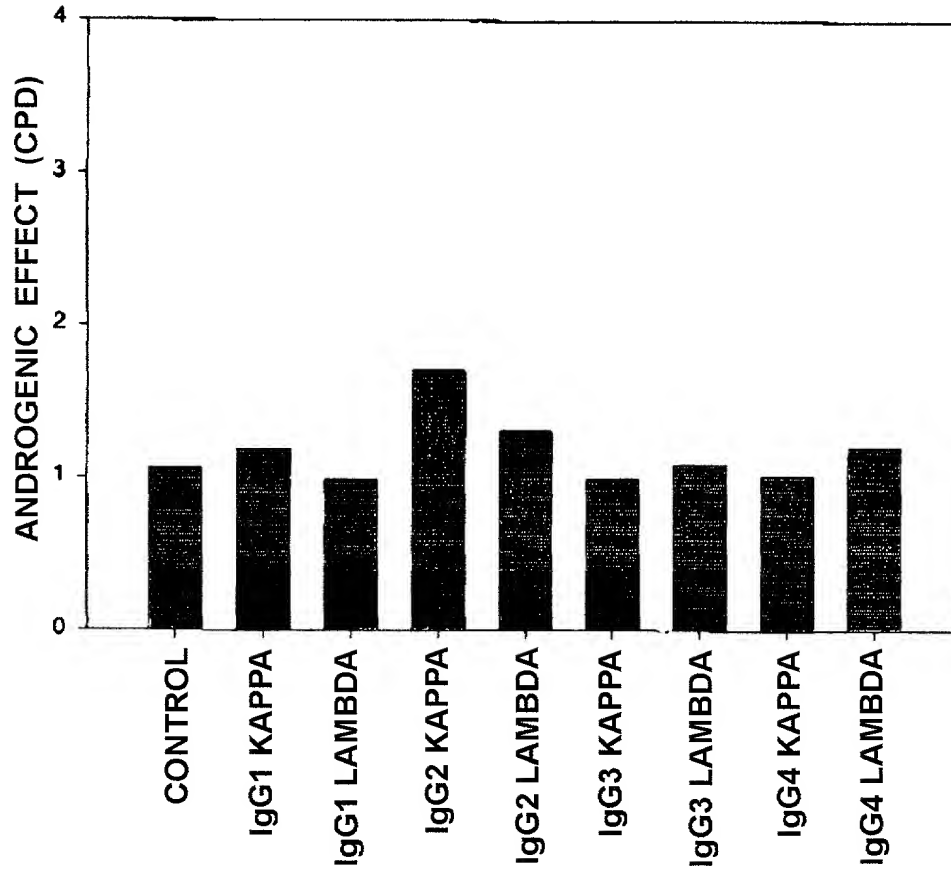
**EFFECT OF IgG ISOTYPES (40 ug/mL) ON LNCaP  
CELL GROWTH IN SERUM-FREE MEDIUM**



**LEGEND:** + = DHT Added  
- = No DHT Added

FIGURE 122

**IgG ISOTYPE ASSAYS WITH LNCaP CELLS IN  
SERUM-FREE DEFINED MEDIUM  $\pm$  DHT**



## FIGURE 123

### MODEL OF EARLY ONSET BREAST CANCER INCLUDING TGF-BETA

#### ER<sup>+</sup> BREAST CANCERS

- (i) Inhibitory receptor(s) for IgA & IgM & IgG1
- (ii) Growth inhibition by IgA & IgM
- (iii) Little or no TGF $\beta$  growth inhibition
- (iv) No TGF $\beta$  receptors



#### NORMAL EPITHELIAL CELLS

- I. Inhibitory receptor(s) for IgA & IgM & IgG1 & TGF $\beta$
- II. Growth inhibition by IgA & IgM & TGF $\beta$

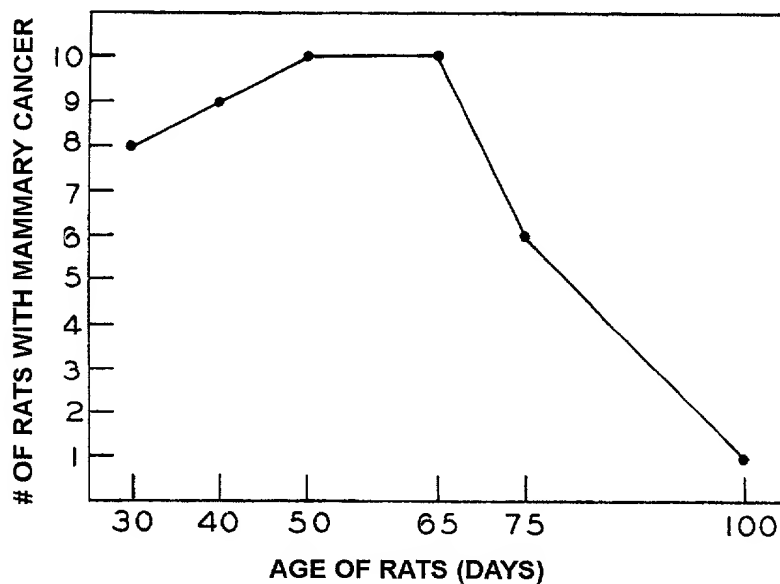


#### ER<sup>-</sup> BREAST CANCERS

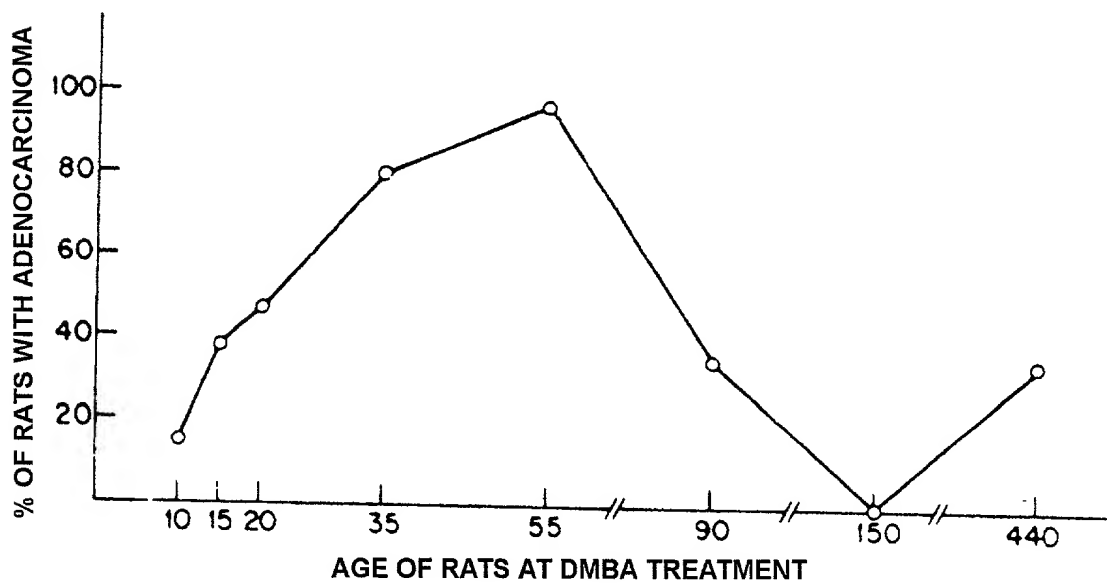
- (i) No functional receptors for IgA or IgM & IgG1
- (ii) No growth inhibition by IgA & IgM
- (iii) High sensitivity TGF $\beta$  growth inhibition
- (iv) TGF $\beta$  receptors present

**FIGURE 124**

**EFFECT OF CARCINOGENS ON MAMMARY TUMOR  
INDUCTION IN RATS OF VARIOUS AGES**



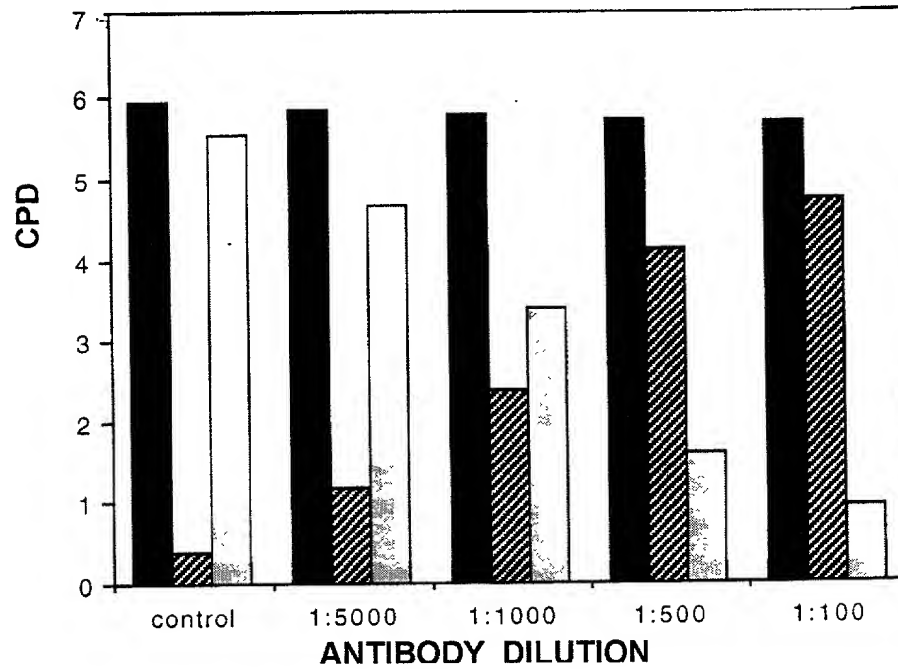
**INCIDENCE OF MAMMARY CANCER IN GROUPS OF 10  
FEMALE RATS OF VARIOUS AGES FED 3-MC, 100 MG**



**INCIDENCE OF MAMMARY ADENOCARCINOMA IN  
RATS GIVEN DMBA AT DIFFERENT AGES**

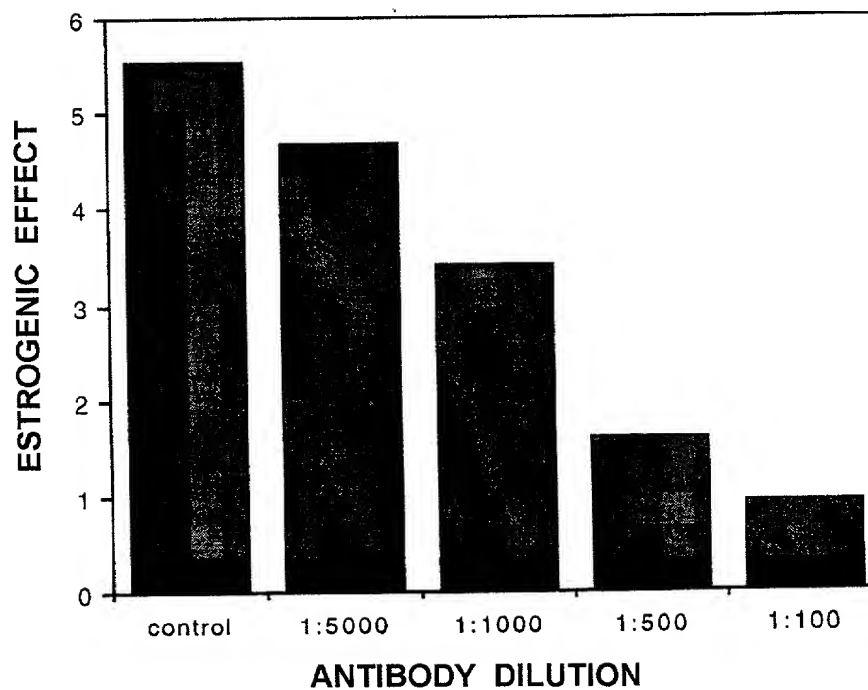
**FIGURE 125**

**ANTI-HUMAN SHBG ANTIBODY IMMUNOPRECIPITATION  
OF THE ESTROGENIC ACTIVITY PRESENT IN  
CDE-HORSE SERUM WITH MTW9/PL2 CELLS**



**LEGEND:**

- = GROWTH IN 50% CDE WITH E<sub>2</sub>
- ▨ = GROWTH IN 50% WITHOUT E<sub>2</sub>
- = E<sub>2</sub> EFFECT



**FIGURE 126**

**ANTI-HUMAN SHBG ANTIBODY IMMUNOPRECIPITATION OF  
THE ESTROGENIC ACTIVITY PRESENT IN CDE-RAT SERUM  
ASSAYED WITH MTW9/PL2 CELLS**

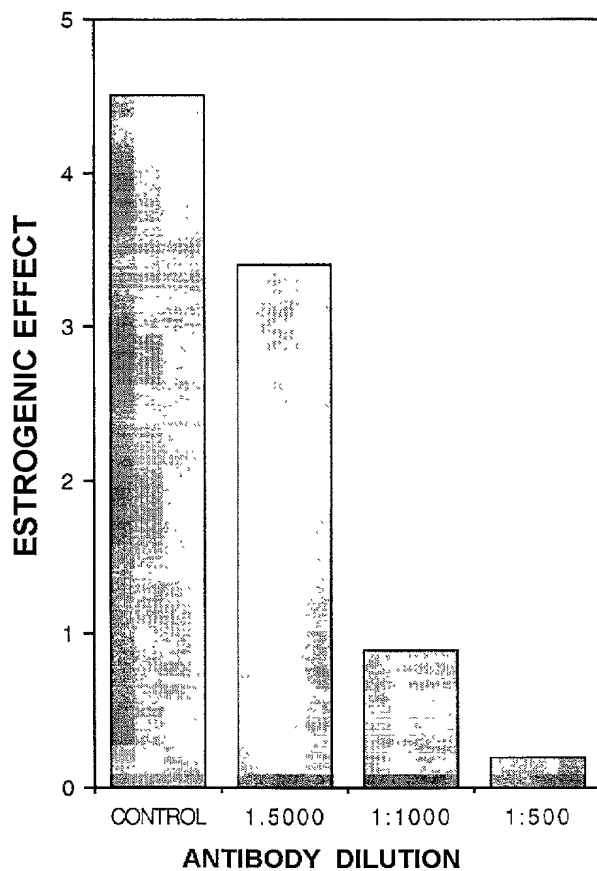
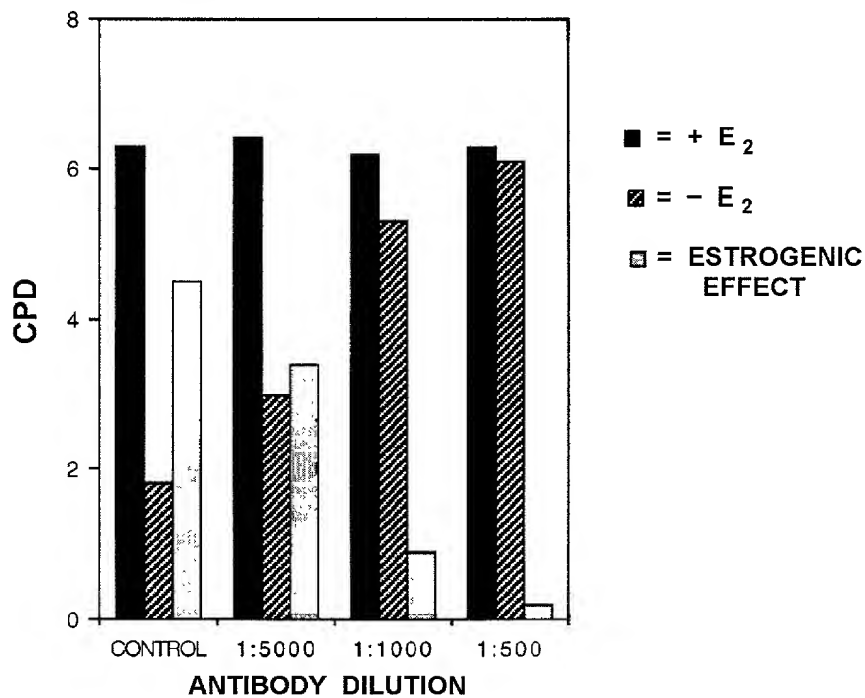
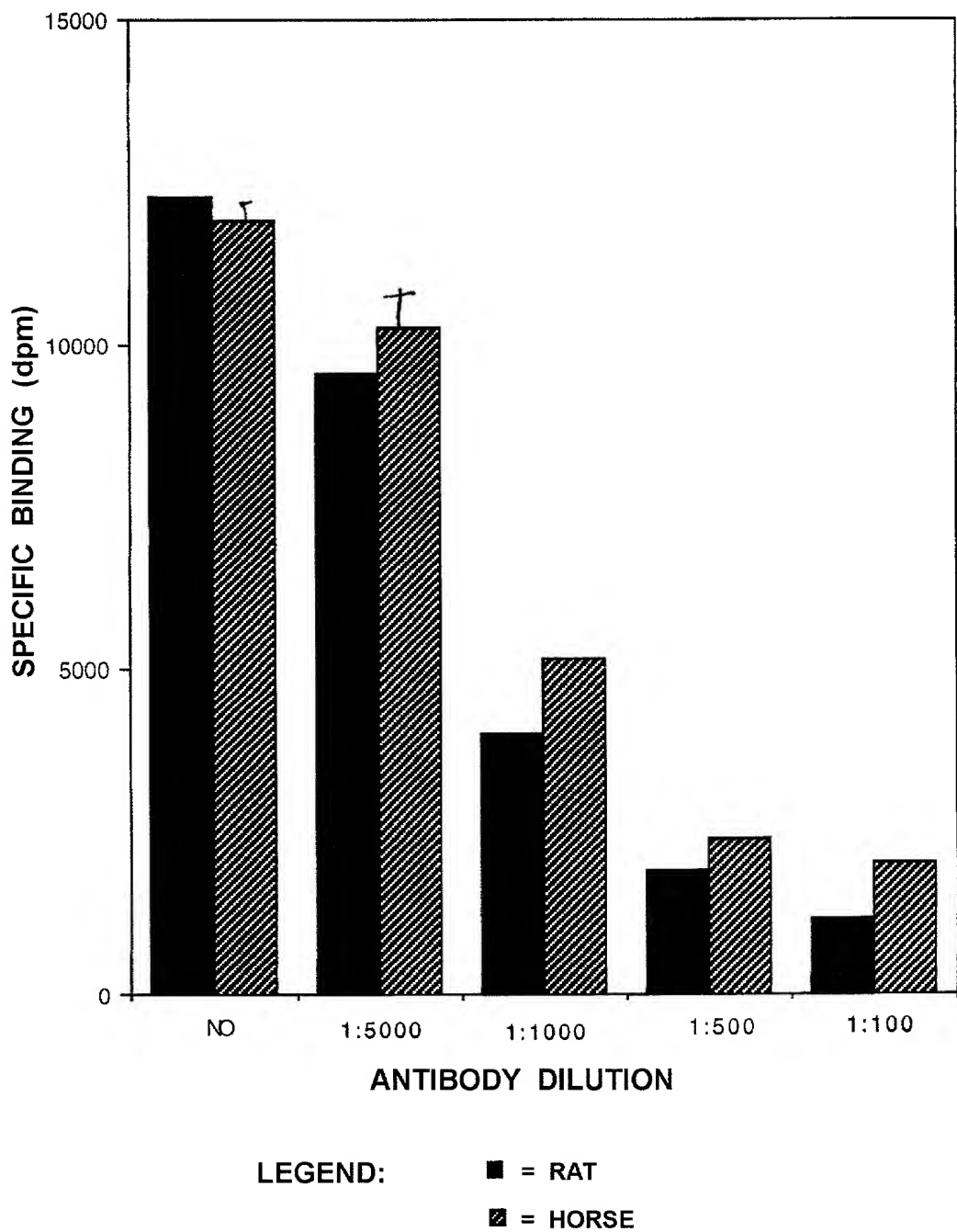


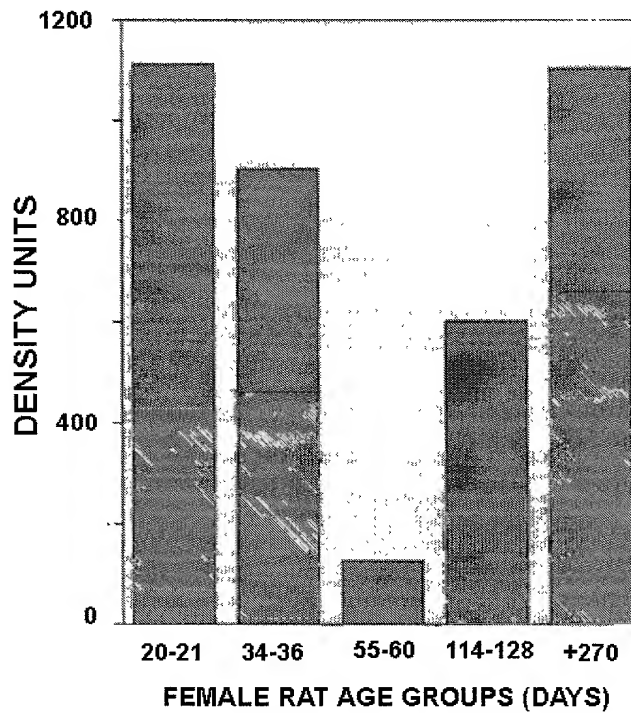
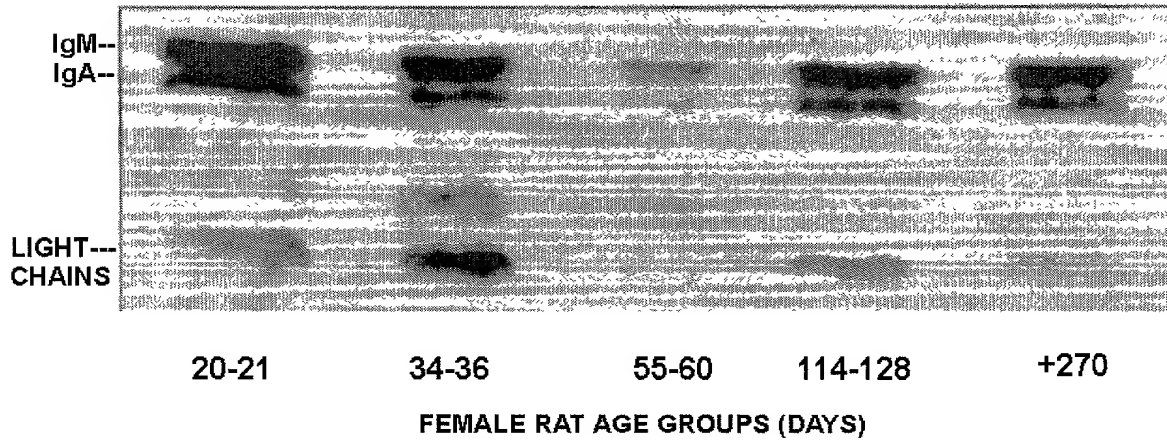
FIGURE 127

ANTI-HUMAN SHBG ANTIBODY IMMUNOPRECIPITATION  
OF THE LABELED STEROID HORMONE BINDING  
ACTIVITY PRESENT IN CDE-RAT SERUM



**FIGURE 128**

**WESTERN ANALYSIS AND DENSITOMETRY OF THE  
IMMUNOGLOBULIN LEVELS IN THE SERUM OF  
FEMALE RATS OF SPECIFIED AGE GROUPS**



**FIGURE 129**

**STRUCTURAL AND FUNCTIONAL ORGANIZATION  
OF THE HUMAN ESTROGEN RECEPTOR- $\alpha$**

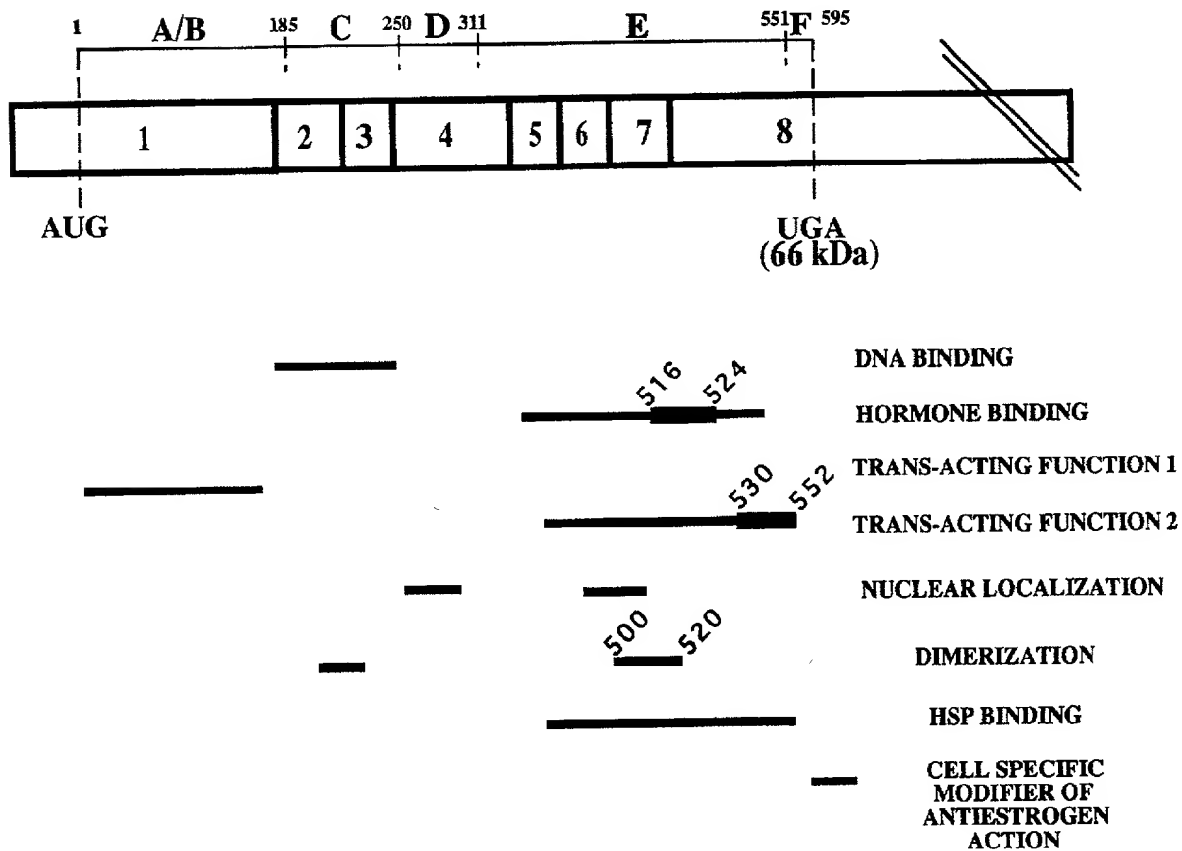
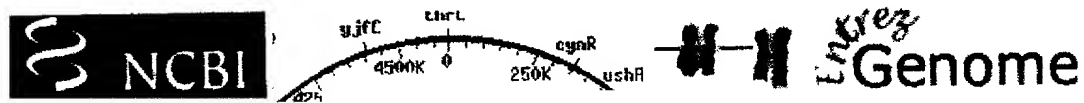
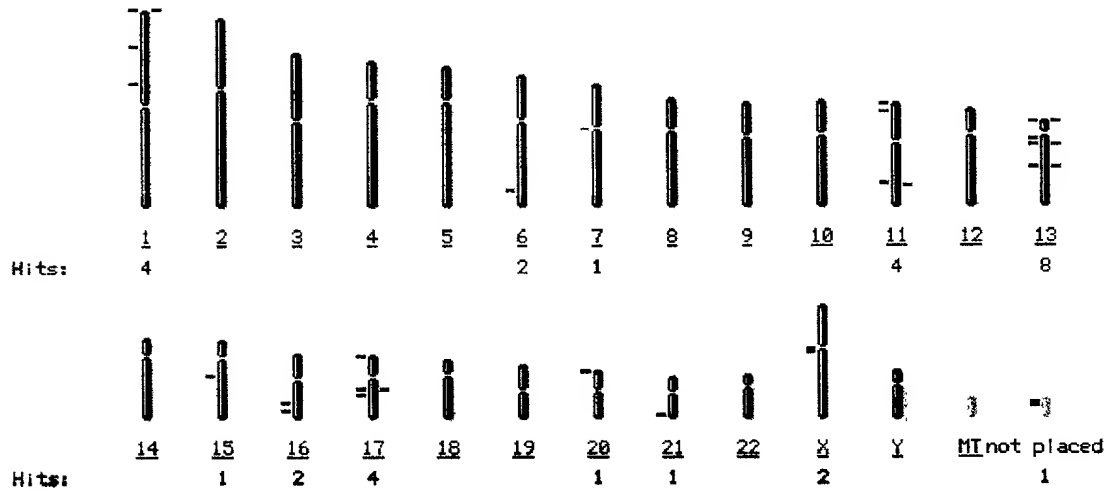


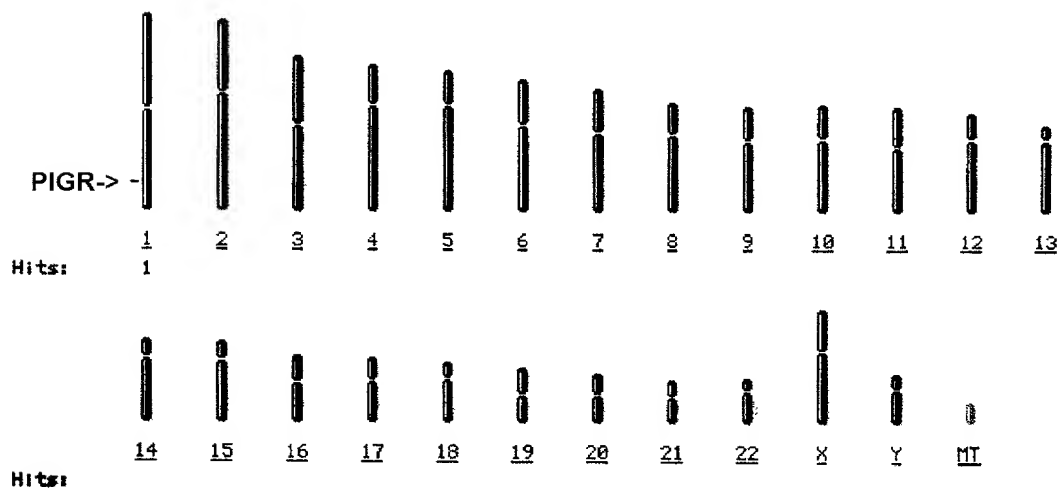
FIGURE 130



"BREAST CANCER" SEARCH - 31 "HITS"



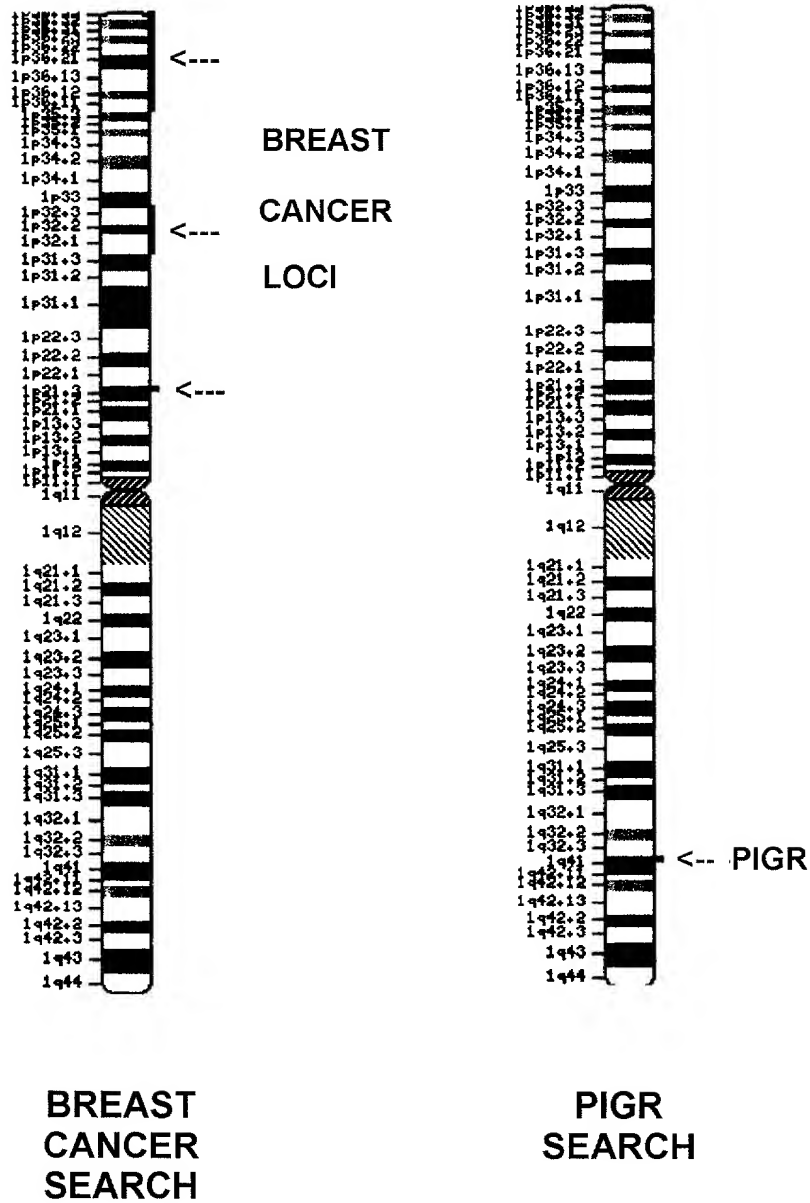
"PIGR" (POLY-Ig RECEPTOR) SEARCH - 1 "HIT"



NOTE: THERE ARE NO BREAST CANCER "HITS" IN THE AREA OF THE POLY-Ig RECEPTOR ON CHROMOSOME 1

FIGURE 131

CHROMOSOME 1



**FIGURE 132**

**CANCER AROUND THE WORLD, 1994-1997  
DEATH RATES PER 100,000 (45 COUNTRIES)**

Country	Colon & Rectum		Breast	Prostate
	Male	Female	Female	Male
United States†	15.2 (27)	10.4 (23)	20.0 (14)	15.9 (20)
Australia‡	20.2 (10)	13.3 (10)	19.9 (15)	19.0 (9)
Austria†	21.7 (8)	12.2 (14)	20.9 (13)	16.9 (14)
Azerbaijan§	6.0 (41)	4.2 (43)	8.6 (42)	5.1 (41)
Bulgaria^	17.2 (20)	11.4 (19)	15.9 (31)	8.5 (34)
Canada‡	16.1 (26)	10.3 (25)	21.5 (10)	16.4 (17)
Chile^	7.0 (38)	6.7 (36)	12.1 (35)	16.0 (19)
China¶^	7.9 (36)	6.4 (37)	5.0 (44)	—
Colombia^	4.8 (44)	5.1 (40)	9.1 (40)	12.6 (28)
Croatia#	22.5 (6)	11.5 (18)	18.5 (20)	13.0 (25)
Cuba‡	9.4 (34)	11.3 (20)	14.9 (33)	20.8 (4)
Czech Republic§	34.3 (1)	17.3 (3)	21.1 (12)	16.0 (18)
Denmark§	22.7 (5)	15.6 (4)	27.6 (1)	19.9 (6)
Estonia§	18.1 (16)	12.2 (13)	18.5 (19)	12.8 (27)
Finland‡	12.1 (31)	8.5 (31)	16.8 (25)	17.6 (12)
France‡	16.6 (22)	9.6 (29)	19.6 (16)	15.8 (21)
Germany†	20.8 (9)	14.0 (7)	21.7 (8)	16.6 (16)
Greece§	8.0 (35)	6.2 (38)	16.2 (27)	9.3 (33)
Hungary^^	34.3 (2)	18.7 (2)	23.7 (6)	18.7 (11)
Ireland‡	22.5 (7)	13.3 (9)	26.1 (2)	18.8 (10)
Israel§	17.9 (18)	13.8 (8)	25.1 (4)	12.0 (30)

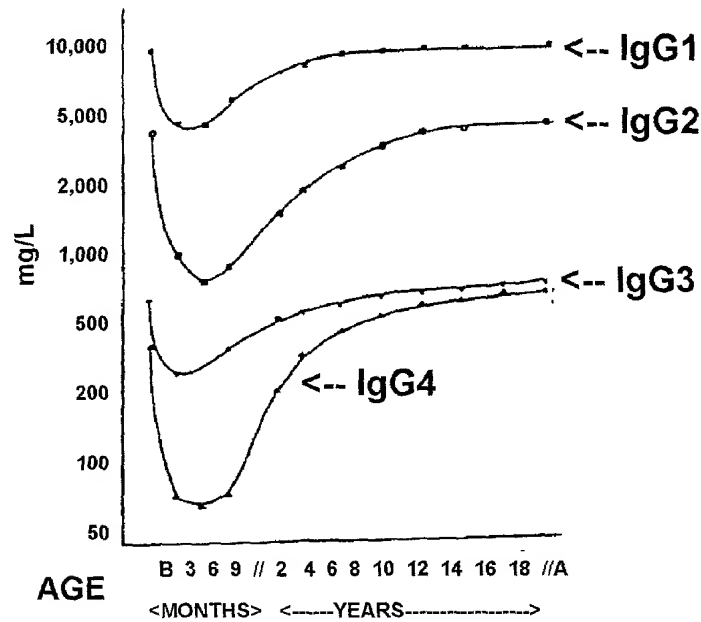
Country	Colon & Rectum		Breast	Prostate
	Male	Female	Female	Male
Japan**	17.1 (21)	9.9 (28)	7.7 (43)	5.1 (42)
Kazakhstan§	12.6 (30)	8.6 (30)	13.2 (34)	5.7 (39)
Kyrgyzstan§	6.9 (39)	4.5 (41)	10.6 (37)	4.3 (43)
Latvia‡	18.3 (12)	11.8 (15)	17.3 (24)	11.5 (31)
Lithuania§	18.2 (13)	11.7 (16)	18.7 (18)	15.2 (22)
Macedonia§	10.8 (33)	7.1 (34)	16.1 (30)	6.2 (38)
Mauritius§	6.0 (42)	3.8 (44)	9.0 (41)	7.7 (36)
Mexico‡	3.6 (45)	3.3 (45)	9.3 (39)	12.8 (26)
Netherlands‡	17.7 (19)	12.7 (11)	26.0 (3)	19.4 (8)
New Zealand^	26.4 (3)	19.1 (1)	22.9 (7)	19.8 (7)
Norway‡	20.0 (11)	14.7 (5)	19.4 (17)	23.2 (2)
Poland§	16.4 (23)	11.0 (22)	16.1 (29)	11.1 (32)
Portugal§	18.1 (15)	10.4 (24)	17.6 (22)	17.2 (13)
Rep. of Moldova‡	16.2 (25)	11.1 (21)	18.2 (21)	5.7 (40)
Romania§	11.3 (32)	7.9 (33)	15.7 (32)	8.3 (35)
Russian Fed.‡	18.2 (14)	12.6 (12)	16.1 (28)	7.2 (37)
Slovakia‡	14.6 (28)	6.8 (35)	—	12.2 (29)
Slovenia§	23.9 (4)	14.0 (6)	21.2 (11)	14.7 (23)
Spain‡	16.4 (24)	10.0 (27)	17.5 (23)	13.9 (24)
Sweden§	13.8 (29)	10.2 (26)	16.8 (26)	21.4 (3)
Trinidad & Tobago^	7.8 (37)	8.3 (32)	21.5 (9)	35.5 (1)
Turkmenistan^	6.2 (40)	4.4 (42)	9.5 (38)	1.4 (44)
United Kingdom‡	18.0 (17)	11.6 (17)	24.5 (5)	16.6 (15)
Venezuela^	5.9 (43)	6.2 (39)	11.8 (36)	20.3 (5)

FIGURES IN PARENTHESES ARE ORDER OF RANK WITHIN SITE AND SEX GROUP

SOURCE: MORTALITY DATABASE 1994-97  
WORLD HEALTH ORGANIZATION, 1999

FIGURE 133

**A: TYPICAL CONCENTRATIONS OF IgG SUBCLASSES DURING CHILDHOOD**



**B: IMMUNOGLOBULIN CHANGES WITH AGE**

